NiPERA INC.
Nickel Producers Environmental Research Association

Lawrence N. Curcio, Ph.D.
President

Dr. C.W. Jameson
National Institute of Environmental Health Sciences
National Toxicology Program
P.O.Box 12233
Research Triangle Park, North Carolina 27709-2233

November 20, 1998

Dear Bill:

As you may recall from my letter to you on November 11, NiPERA promised to have comments to NTP on the Draft RoC Background Document for Nickel Compounds to you no later than November 30. We are extremely pleased to be able to provide them to you for distribution to the subcommittee of technical experts today. I recognize that in our telephone call the other day you had indicated that NTP would not be considering nickel metal in its listing of carcinogens. You will notice however, that we do refer to metallic nickel in these comments primarily because the draft RoC Background Document refers to metallic nickel. I think, if anything, our comments show scientific support for the conclusion already reached by NTP on nickel metal. The main point of our comments however, is to put our concerns with the Draft RoC document into a format that provides for increasingly greater levels of detail which would hopefully match the needs of the various experts on your subcommittee:

- A two-page Executive Summary
- Several pages of more specific comments and concerns with items and statements included within the Draft RoC document.
- A focused conclusion emphasizing the differences in carcinogenic potential between the various nickel compounds.
- Several references that provide support for the NiPERA comments which were not cited in the RoC review.
- Finally, four appendices providing very detailed comments on various manuscripts used in the support document, as well as additional information on nickel manufacturing operations.

We very much appreciate the cooperation and transparency with which the NTP has chosen to work with NiPERA on the evaluation of nickel compounds. Please do not hesitate to contact me if you have any further questions. I remain,

Sincerely yours,

2605 Meridian Parkway, Suite 200, Durham, NC 27713
Telephone 1-919-544-7722 • Fax 1-919-544-7724

Comments of the Nickel Producers Environmental Research Association on the National Toxicology Program Draft RoC Background Document for Nickel Compounds

November 20, 1998

Same enclosure as w/ NIPERA 1.29.99 Letter.

1. Executive Summary

The U.S. National Toxicology Program (NTP) is reviewing the database on the potential carcinogenicity of nickel and nickel compounds. In the NTP's Eighth Report on Carcinogens, *Nickel and Certain Nickel Compounds* (*i.e.*, not including water soluble nickel compounds) were listed as substances that are "*reasonably anticipated to be a carcinogen*". The new proposal for the Ninth Report on Carcinogens would list *Nickel and Nickel Compounds* as substances that are "*known human carcinogens.*" NiPERA believes that this change would be scientifically unjustified and inappropriate. NiPERA's major objection to the NTP's proposal to list *Nickel and Nickel Compounds* as "*known human carcinogens*" in the Ninth Biennial Report on Carcinogens is that it fails to recognize the **differences in the carcinogenic potential of the various forms of nickel.**

The NTP proposal to list *Nickel and Nickel Compounds* as substances that are "*known human carcinogens*" is presumed to be based on the information contained in the Draft RoC Background document. This draft document concludes that "*nickel ion is a human carcinogen and all compounds that contain nickel ions should be considered human carcinogens*". Based on these conclusions, the NTP proposal would appear to be fully justified. However, a closer look at the Draft RoC Background document reveals that its conclusions are based on a less than objective selection, presentation, and interpretation of the data for nickel and its compounds. The consistently low quality of the data presentation and interpretation is particularly alarming in view of the excellent analyses presented by the NTP in its discussions of the toxicology of nickel compounds included in the NTP Technical reports for nickel subsulfide, high temperature [green] nickel oxide, and nickel sulfate hexahydrate (NTP, 1996a-c).

One of the main problems found in this document relates to the selection, presentation, and interpretation of the epidemiological, animal and *in vitro* data pertaining to soluble nickel compounds.

- In some cases, isolated studies are discussed without making any attempts to integrate the data from one study with the rest of the available data. In many cases, the reporting is superficial and incomplete, in other cases, the reporting is just wrong.
- A discussion of the epidemiologic studies in which exposures to soluble nickel compounds occur in refinery workers should take into account the fact that all cohorts had mixed exposures to more insoluble nickel compounds and to other confounders (*e.g.,* acid mists, arsenic, chromium, cigarette smoking). In addition, studies of platers (almost exclusively exposed to soluble nickel compounds) should be included in the discussions. Finally, an effort should be made to look at the consistency of the data from all studies in assessing the human carcinogenic potential of soluble nickel compounds.
- Two rat studies by intraperitoneal route of exposure are featured prominently in the report while a dozen other negative studies (including relevant routes of exposures such as the inhalation NTP 1996a bioassay) are ignored.
- The significance of these two intraperitoneal studies for the carcinogenic assessment of soluble nickel compounds can be seriously questioned given the very unique conditions under which rats developed renal tumors (only males with concurrent exposure to sodium barbital) and the high toxicity experienced by the pups with pituitary tumors in the transplacental carcinogenicity study.

A second significant problem is the lack of understanding of the mechanistic data pertaining to the carcinogenicity of certain nickel compounds.

- It is not just the presence of nickel in any compound that will determine the positive respiratory carcinogenic potential of the compound but rather the availability at nuclear sites within the target epithelial cells of the lung or nose of the nickel ion released from this compound.
- A nickel-containing compound that is highly insoluble may not cause tumors because even if particles are endocytized by the epithelial cells, not enough nickel ions will reach the nucleus.

- On the other extreme, a nickel compound that is completely soluble will be cleared from the nose and lung very quickly (no accumulation), will not be able to enter the cell by endocytosis, and will not be available at nuclear sites of target cells (see NTP 1996 bioassay).
- Only those nickel compounds (*e.g.*, nickel subsulfide) that are readily endocytized by epithelial cells, have intermediate clearance rates, have increased solubility under acidic endocytic pH, will result in sufficient amount of nickel ions at nuclear sites to induce tumors (see NTP 1996b bioassay). The overall human, animal and mechanistic data are consistent with this interpretation but are not consistent with all nickel compounds being human carcinogens.

The NTP Draft RoC Background Document concludes that *nickel and all nickel compounds should be human carcinogens* based on biased an inaccurate data discussions. **NiPERA strongly urges NTP to undertake a revision of the NTP Draft RoC Background Document, including a careful re-analysis of the available data and elimination of obvious errors, before this document is used as a background document to evaluate the carcinogenic potential of nickel compounds.** The NTP technical reports with nickel compounds (NTP, 1996) should be used as a model since they present a more thoughtful data integration and critical examination of the many animal and human studies. In addition, the NTP Technical reports display a significant understanding of the mechanistic data, an area that was quite misunderstood in the NTP Draft RoC Background Document.

A more thorough examination of the *in vitro*, animal, and epidemiologic data pertaining to commercially relevant nickel compounds[1] will reveal that these compounds have very different biological behaviors, particularly with regard to respiratory carcinogenicity (see the October 13, 1998 NiPERA comments on the NTP proposal for Classification of Nickel and Nickel Compounds).

[1] The classes of nickel compounds discussed in this paper are: metallic nickel, oxidic nickel (including nickel oxides, hydroxides, silicates, carbonates, and complex nickel oxides), sulfidic nickel (including nickel sulfide and subsulfide), water soluble nickel compounds (including hydrated forms of nickel acetate, sulfate, chloride, *etc.*), and nickel carbonyl. Metallic, oxidic, and sulfidic nickel compounds and nickel carbonyl are insoluble in water.

Table of Contents

PAGE

2. Comments Arranged by Page and Document Section

2.1. CARCINOGENICITY

FIRST PAGE (NOT NUMBERED), PARAGRAPH 1:
The NTP Draft RoC Background Document concludes that all nickel compounds should be classified as "*...known to be human carcinogens based on increased risk of cancer in workers and evidence of malignant tumor formation by multiple routes of exposure, at various sites, in multiple species of experimental animals.*" This statement is flawed in that consistent human and animal data showing increased carcinogenicity (at several sites and by several routes of exposure) are available for only one nickel compound: sulfidic nickel including nickel subsulfide. For all other nickel compounds (nickel carbonyl, oxidic nickel and soluble nickel compounds) the statement is not true and does not even agree with the data reviewed in this report.

The NTP Draft RoC Background Document states that because all "*...nickel compounds act by the generation of nickel ions at critical sites in target cells all these compounds can be evaluated as a single group.*" This statement is false and it appears to be based on the limited consideration of a subset of animal studies with disregard for the results demonstrated in other studies (including the NTP 1996a-c studies). The consideration of all nickel compounds as a single group for carcinogenic evaluation demonstrates a lack of understanding of the mechanistic and toxicokinetic data related to nickel compounds.

It is not just the presence of nickel in any compound that will determine the positive respiratory carcinogenic potential of the compound but rather the availability at nuclear sites within the target epithelial cells of the lung or nose of the nickel ion released from this compound. A nickel-containing compound that is highly insoluble may not cause tumors because even if particles are endocytized by the epithelial cells, not enough nickel ions will reach the nucleus. At high concentration, a highly insoluble nickel compound may cause tumors only secondary to a particle effect. On the other extreme, a nickel compound that is completely soluble will be cleared from the nose and lung very quickly (no accumulation), will not be able to enter the cell by endocytosis, and will not be available at nuclear sites of target cells due to rapid binding to cytoplasmic proteins (see NTP 1996 bioassay). Only those nickel compounds (*e.g.*, nickel subsulfide) that are readily endocytized by epithelial cells, have intermediate clearance rates, have increased solubility under acidic endocytic pH, will result in sufficient amount of nickel ions at nuclear sites to induce tumors (see NTP 1996b bioassay). The overall human, animal and mechanistic data are consistent with this interpretation but are not consistent with <u>all</u> nickel compounds being human carcinogens.

FIRST PAGE (NOT NUMBERED), PARAGRAPH 2:
NTP should remember that IARC's pronouncements in 1990 were not based on the datasets available today, therefore pronouncements that may have been justified at the time need to be re-evaluated considering the current body of data. More recent assessments by ACGIH for example have taken speciation of nickel compounds into account for carcinogenic classification.

Of all the categories of nickel compounds, the need for speciation is most compelling for soluble nickel compounds. There are a large number of negative animal carcinogenicity studies by relevant routes of exposure, starting with the well-conducted NTP inhalation bioassays in mice and rats (NTP 1996c) and continuing with five negative oral studies in mice, rats, and dogs (Schroeder *et al.*, 1964; Schroeder *et al.*, 1974; Schroeder and Mitchner, 1975; Ambrose *et al.*, 1976; Kurokawa *et al.*, 1985). Even a non-relevant route of exposure like intramuscular injection gave negative results (Gilman, 1962; Payne, 1964; Kasprzak *et al.*, 1983; Kasprzak, 1994; in rats). In an intraperitoneal study, the administration of a soluble nickel compound by itself was also negative (Kasprzak *et al.*, 1990). In that study, administration of the non-genotoxic carcinogen sodium barbital resulted in kidney tumors in male rats (only). When the soluble nickel compound was administered with sodium barbital, a higher number of kidney tumors (in male rats

only) were induced (Kasprzak *et al.*, 1990; Diwan *et al.*, 1992). This phenomenon was later explained by the enhanced susceptibility of male kidneys to the sodium barbital effects (possibly involving the α-2 microglobulin mechanism). EPA and other regulatory agencies agree that these type of tumors should not be considered in carcinogenicity assessment.

Therefore, out of 14 animal studies, there is only one positive study, by one route of exposure, in one animal species with a soluble nickel compound. This study is a transplacental rat carcinogenicity study in which dams were injected intraperitoneally with a soluble nickel compound and the surviving pups were examined for tumors. A significant fraction of both male and female pups developed pituitary tumors (Diwan *et al.*, 1992). In the context of a dozen negative studies, the relevance of one transplacental study for the carcinogenic assessment of soluble nickel compounds should be seriously questioned. This is particularly true based on fact that the study used an irrelevant route of exposure for risk assessment, the study caused high toxicity resulting in 88% mortality, and the fact that this tumor type has never been observed in any other animal study (even those that used a clearly carcinogenic nickel compound such as nickel subsulfide) or in human studies (+50,000 workers). The transplacental study of Diwan *et al.* is discussed further in comments provided under Section 4 and in Appendix A.

The overwhelmingly negative animal data, together with the epidemiological data that suggests an enhancing rather than a direct carcinogen role for soluble nickel compounds, does not justify the classification of soluble nickel compounds as *Known to be Human Carcinogens.*

FIRST PAGE (NOT NUMBERED), PARAGRAPH 2:
With regard to the human epidemiologic data pertaining to soluble nickel compounds, the document wrongly states that exposure to soluble nickel alone in a refinery resulted in excess lung and nasal cancers. Such a refinery cohort does not exist and as mentioned in Andersen *et al.* (1996), workers always had mixed exposures to soluble and insoluble nickel compounds, arsenic, acid mists, *etc.* There are however, smaller nickel plater cohorts that have exposures almost exclusively to soluble nickel compounds and show no excess risk of respiratory tumors (Burges *et al.*, 1980; Pang *et al.*, 1996). Unfortunately, these references were totally left out of the NTP Draft RoC Background Document. [See further comments on this issue under Section 3.2.4.]

2.2. OTHER INFORMATION RELATING TO CARCINOGENESIS OR POSSIBLE MECHANISMS OF CARCINOGENESIS

SECOND PAGE (NOT NUMBERED), PARAGRAPH 2:
At the end of this section, the intraperitoneal transplacental study by Diwan and coworkers is featured again as evidence of the carcinogenicity of soluble nickel compounds while the recent negative inhalation studies by the NTP, which used a relevant route of exposure and two animal species, are not even mentioned. Furthermore, the document gives great relevance to the kidney tumors seen only in males, and only after sodium barbital exposure. At the same time, the NTP Draft RoC Background Document ignores all the information pertaining to the association between the nongenotoxic carcinogen, sodium barbital, and the kidney tumors in seen only male animals (possibly involving the α-2 microglobulin mechanism). The document also fails to mention that soluble compounds alone did not cause tumors while sodium barbital alone did.

2.3. IDENTIFICATION AND CHEMICAL-PHYSICAL PROPERTIES OF NICKEL COMPOUNDS

PAGE 1-1, SECTION 1.0, PARAGRAPH 1:
In line 1 the word "*thousands*" should be changed to "*many*" since the number of nickel compounds is on the hundreds rather than thousands range. The list of compounds with potential for occupational exposure (Table 1-1) actually has less than 80 compounds.

PAGE 1-1, SECTION 1.0, PARAGRAPH 1:
In line 5 "*melting*" should be added along with "*fabrication and joining*" since the risk of exposure to nickel is important during this stage of processing.

PAGE 1-1, PARAGRAPH 5:
The NTP Draft RoC Background Document states categorically: "*It is expected that ionic nickel may arise from any nickel compound at physiological pH.*" This is incorrect given that one nickel compound listed in Table 1-1 of the NTP Draft RoC Background Document itself is stated to "*dissolve in hot sulfuric or nitric acid only.*" Clearly no physiological pH on this planet will dissolve this compound. In fact, since the solubilization or corrosion of nickel ions from a nickel compound particle is important in eliciting a biological response, it should be noted that solubilization/corrosion of a nickel compound is directly relevant to the route of exposure and the appropriate subcellular organelle. Sweeping generalizations of this type should be avoided.

TABLE 1-1:
The information in Table 1-1 (pages 1-2 to 1-10) is reproduced from various sources and assumed to have been accurate copied. However, the formula for Raney Nickel, which is the last entry in the Table, should be "nickel", it is just porous nickel.

2.4. HUMAN EXPOSURE

PAGE 2-1, SECTION 2.1, PARAGRAPH 1:
The corresponding sentences in this paragraph should be corrected as follows:

> *"In the United States, consumption reached about 200,000 tons per year (U.S. Bureau of Mines, 1991; cited by NTP, 1996), but has recently fallen to the 180,000 ton level. The use of primary nickel can be divided into six sectors: stainless steel, alloy steel, nickel alloys, electroplating, foundry and other. In 1996, approximately 73% of primary nickel was used for the production of stainless and alloy steels, 14% went into nonferrous and superalloys, 9% for electroplating, 3 % into foundry products and the balance of 2% was used in other applications such as, chemicals, catalysts, batteries, pigments and ceramics (Kuck, 1997a; NiDI, 1997)."*

PAGE 2-1, SECTION 2.2, PARAGRAPHS 2 AND 3:
For clarification the corresponding sentences in these paragraphs should be corrected as follows:

> *"Metallic nickel is produced from sulfide ores and oxide (laterite) ores. The oxide ores are found in tropical regions and areas that were once considered tropical such as parts of the Pacific Northwest. Neither type of ore currently processed averages more than 3% nickel (Warner, 1984; cited by IARC, 1990). Nickel and co-products are recovered from sulfide ores by a combination of flotation, roasting, smelting, electrolysis or decomposition processes. (IARC, 1990). Nickel is recovered from oxide ores by either hyrometallurgical or pyrometallurgical techniques (IARC, 1990). Other ways of obtaining nickel units are through the recycling process, consumer scrap, and as a by-product from the refining of other metals such as copper and platinum (Sibley, 1985; cited by IARC, 1990).*
>
> *Nickel products are broadly classified by the amount of nickel they contain. Class I products are defined as containing > 99.8% nickel, whereas Class II products vary in their nickel content (NiDI, 1997). Class I nickel products are refined using a variety of processes which decrease impurities such as antimony, cobalt, arsenic, zinc, copper, iron and lead. Cobalt closely resembles the physical and chemical properties of nickel and is often difficult to remove completely from the mined ores, therefore many Class I products may contain minor amounts of residual cobalt. Nickel products designated as Class II material such as nickel oxide, metallized oxide and ferronickel are produced directly by hydrometallurgical or pyrometallurgical techniques and are*

sufficiently pure to be used without further refining in applications like stainless steel production (Ullman, 1985)."

PAGE 2-2, TABLE 2-1:
The section on the uses of "Nickel" should be replaced with the following:

"Wrought and cast stainless steels, alloy steels, cupronickels, superalloys, electroplating, magnets, coinage, catalysts, batteries, electrical contacts, and electrodes, pigments."

PAGE 2-5, SECTION 2.2.1, PARAGRAPH 4:
For clarification, the second line in this paragraph should read as follows:

"...demand for primary nickel increased significantly in 1995 when it rose by 15%..."

and the following sentence should be added after (Kuck, 1997b).

"Current consumption is near the 1 M ton mark."

PAGE 2-5, SECTION 2.3.1, PARAGRAPH 5:
In the second sentence *"mechanically"* should be replaced by *"by flotation"* while in the third sentence *"is"* should be replaced by *"maybe."*

PAGE 2-5, SECTION 2.3.1, PARAGRAPH 5:
Delete the last line "*Lateritic ores may be...*" It is repeated on the top of page 2-6.

PAGE 2-6, SECTION 2.3.1, PARAGRAPH 3:
For clarification starting at the first line, the paragraph should be modified to read, "*...a nickel-copper matte. The nickel is leached from the matte and recovered by electrolysis of the solution. The atmospheric.....nickel and cobalt in the feed. A series...*"

PAGE 2-6, SECTION 2.3.2, PARAGRAPH 4:
In the third line *"most"* should be replaced by *"over half."*

PAGE 2-8, SECTION 2.3.3, PARAGRAPH 2:
Delete first sentence starting with "*Table 2-2 is.....*" and add the following sentence at the end of the paragraph:

"Table 2-2 gives a summary of the current producers of refined nickel and indicates the type of material processed, the process technology used, and the nickel products produced."

PAGE 2-9, TABLE 2.2:
This Table is not complete. A substitute Table is enclosed in Appendix B which lists all current producers, including a brief description of the type of material they process, the main process technology and the products made. No reference is made to the specific types of nickel-bearing materials involved in processing since the chemistry is complex and would require a very detailed analysis. It would be virtually impossible to summarize all the materials used in a table. Facilities no longer in production have not been included.

PAGE 2-10, SECTION 2.4.1:
The average levels of nickel found in the ambient air ought to be reported. As noted in the document, they are very low (much lower than most of the occupational values reported in Table 2-3). In as much as inhalation is the main exposure route of concern regarding the health effects of nickel, the reader should understand that health risks due to the inhalation of ambient nickel will likely be negligible given the minute amounts of nickel present in the air (see later comments regarding such risks).

PAGES 2-10, SECTION 2.4.2:
It is not clear why mining, milling, smelting, and refining should be considered among the most relevant industrial sectors with respect to this document. The NTP guidelines require that this document be focused on the United States. There are no nickel mining, milling, smelting or refining operations in the U.S. Even in the past, the presence of nickel production operations in the US has been very limited. US operations where nickel is potentially present are confined to using-industries where exposures will mainly be to oxidic, metallic, and soluble nickel. Furthermore, the oxidic nickel exposures in using industries tend to be different from the oxidic nickel exposures that were associated with the nasal and lung cancers seen in the past in producing industries. No respiratory cancers have been associated with exposures to nickel-using industries. This is an important point that ought to be elaborated on in later sections of the NTP Draft RoC Background Document.

PAGE 2-12, SECTION 2.4.2, PARAGRAPH 1:
"Table 2-1" should be cahnged to "Table 2-4."

PAGE 2-14, SECTION 2.5:
The NIOSH REL is dated (1977) and was proposed long before scientists knew much about the health effects and cancer mechanisms of nickel and individual nickel compounds. NIOSH has not been active in researching the health effects of nickel, nor in up-dating its recommendations. Either the REL should not be reported or should only be mentioned with appropriate qualifying statements regarding its obsolescence.

PAGE 3-1, SECTION 3.1, PARAGRAPH 2:
The second and third sentences of this paragraph have been taken out of context from an occupational criteria document that was prepared by a group of independent scientists for the Directorate General V of the CEC.[2] These sentences are not generic to all oxidic and sulfidic cancer incidences and refer only to a group of refinery workers employed at Falconbridge's Kristiansand, Norway operation. In addition, it appears that the reference to Ni-Cu oxides and impure NiO has been confused. Exposure levels at a nickel refinery in Clydach, Wales were estimated at 1-10 mg Ni/m^3 (mainly as Ni-Cu oxides) prior to 1936 and 1-5 mg Ni/m^3 (mainly as impure NiO) in subsequent years. Exposures at Kristiansand were mainly to Ni-Cu oxides.

PAGE 3-1, SECTION 3.1, PARAGRAPH 3:
The end of paragraph three would be a useful place to integrate the data presented under Section 2.4.1. The conclusions of Steenland *et al.* (1996, cited in the text, but missing from the references) are much the same as the ICNCM which noted that "*the risk to the general population from exposure to the extremely small concentrations [of nickel] (less than 1 µg Ni/m^3) to which it is exposed in the ambient air is minute, if indeed, there is any risk at all.*"

2.5. STUDIES POST IARC (1990)

PAGE 3-2, SECTION 3.2.1:
The Moulin *et al.* (1990) study of stainless steel and ferrochromium production workers is essentially a negative study for nickel. While elevated odds ratios were seen for nickel and/or chromium workers in a nested case control study (OR=3.4 and OR=2.75), they were not statistically elevated. In contrast, the ORs for workers definitely or possibly exposed to PAHs were 4.51 and 14.86, respectively. These ORs were statistically raised. These results agreed with the significantly high SMRs observed in the case of people hired during the early years of the plant when PAH pollution was likely to be at its highest in the

[2] This document has elsewhere been cited as "NiPERA, 1996". However, it should be noted that this document, which was a Criteria Document on Nickel and its Compounds, was authored by a group of independent scientists for DGV (Drs. Agius, Crawford, Goyer, Hewitt, Mark, Rappaport, Skopek, Templeton, Vincent, and Zatka). NiPERA served purely in a coordinating and editing capacity.

ferrochromium workshops. The authors of the study concluded that their findings clearly suggested that the excess of deaths from lung cancer seen in the cohort (no nasal cancers were observed) was attributable to former PAH exposures in the ferrochromium production workshops rather than to exposures in the stainless steel manufacturing areas. Similar attributions of cancer risk to PAH exposures have been seen among nickel/copper smelter and refinery workers in Sudbury (Verma *et al.*, 1992). In a second study by Moulin *et al.* (1992), in which data from a second factory was added to the original study, the overall SMR for lung cancer, again, was not significantly raised at 130 (95% CI 94-175).

PAGE 3-3, SECTION 3.2.3:
It would be worth noting in the discussion of the Simonato *et al.* (1991) study, that the complex nickel oxides found in welding stainless steel do not contain copper. This is important because one of the predominant theories for the existence of lung and nasal cancer in nickel refinery workers in the past was due to their exposure to nickel-copper oxides, per se, rather than other complex nickel oxides. Lack of evidence of excess cancer deaths in workers in nickel-using industries (*e.g.* stainless steel and high nickel alloy workers) and producing industries where exposures were predominantly to silicate oxides or complex nickel oxides free of copper (New Caledonia, Oregon) lends credence to this theory. Only in workers who were exposed to high concentrations of nickel-copper oxides have excess respiratory cancers been seen.

PAGE 3-4, SECTION 3.2.4:
The two studies regarding the Finnish nickel refinery workers (Karjalainen *et al.*, 1992; Anttila *et al.*, 1998) and the up-date of the Norwegian refinery workers (Andersen *et al.*, 1996) require additional analysis from that which has been presented in this document. The interpretation and discussion of the epidemiologic findings, particularly with respect to soluble nickel exposures, are oversimplified. In particular, the papers inadequately discuss several factors that argue against the authors' conclusions that soluble nickel is mainly responsible for the elevated respiratory cancer risks in these cohorts. As indicated by the discussion that follows, the author's conclusions are largely speculative, as alternative hypotheses for the observed respiratory cancer risks are equally plausible.

The Norwegian Study

It is true that excess lung and nasal cancer risks were observed among Kristiansand workers in the electrolysis department exposed mainly to soluble nickel (ICNCM, 1990; Andersen *et al.*, 1996). However, it should be noted that insoluble nickel was also present. In fact, it was the presence of greater amounts of insoluble nickel at Kristiansand that was believed to account for the differences seen in cancer risks between electrolysis workers at Kristiansand and Port Colborne, Sudbury (ICNCM, 1990). Both groups of workers were exposed to approximately similar concentrations of soluble nickel (they were slightly higher at Kristiansand), but insoluble nickel concentrations at Kristiansand were seven times those at Port Colborne. Only the Kristiansand workers developed excess lung cancers.[3] The conclusions reached by Andersen *et al.* in their 1996 follow-up of the cohort, therefore, are not materially different from the ICNCM conclusions. This would be expected in as much as most of the cohort was hired prior to 1960 and a considerable amount of the follow-up in the latter study (at least 24 years) had already occurred at the time the cohort was studied by the ICNCM.

What is unfortunate in the up-dated study is that, while Andersen *et al.* noted the association of lung cancer with soluble nickel exposures, they failed to thoroughly explore the likely role of soluble nickel acting indirectly as a promoter of lung cancer in cigarette smokers. Indeed, the most import new information to be derived from the Andersen follow-up is the prominent role that cigarette smoking played in the lung cancers seen at Kristiansand. A distinctly synergistic

[3] With respect to nasal cancers, Andersen *et al.* concluded that the evidence for linking nasal cancer to oxidic nickel exposures was much stronger than it was for soluble nickel. Further, no new nasal cancers have occurred in Kristiansand workers first employed since 1956, strongly suggesting that the nasal cancer cases seen in this study were linked to the early mixed exposures of insoluble and soluble nickel at relatively high concentrations.

lung cancer response between smoking and exposure to the mixture of soluble and insoluble nickel compounds that the workers were exposed to was observed. In the small number of nickel-exposed workers who did not smoke, there was no evidence that nickel exposures increased the risk for lung cancer (see Appendix C for further comments on this study). A similar lack of excess respiratory cancers was noted in a 1996 cancer mortality study in a relatively small population of nickel platers exposed solely to nickel chloride and sulfate mists (Pang *et al.*, 1996). This study should be included in the NTP Draft RoC Background Document.

The results from the above studies are in good agreement with the original theory advanced by the ICNCM that the role of soluble nickel was likely the enhancement of the carcinogenicity of other agents present, including insoluble nickel compounds and, as strongly suggested by the recent Andersen study, cigarette smoking.

The Finnish Studies

In the Finnish refinery studies, three nasal cancer cases were identified and a 2-fold increase in lung cancer risk was found in nickel workers with more than 20 years employment. While these cancers have been attributed to soluble nickel exposures at fairly low levels, this claim is not well-supported.

First, it is questionable whether the "low-levels" of soluble nickel reported in these studies are pertinent to the analyses of the respiratory cancers seen in these workers. The use of 1979-1980 exposure measurements (reported to be below 0.5 mg Ni/m^3) as the basis for the analyses of cancers that were likely induced in the 1960s (particularly the nasal cancers) is questionable. Data available from the company suggest that earlier exposures--not only to soluble nickel, but also insoluble nickel and acid mists containing sulfuric acid--may have been higher. Technological changes purposely implemented to lower exposures prior to 1980 bear this out (see appendix).

Second, in the case of the lung cancers, smoking data are unavailable for these workers. As indicated in the above study on Norwegian electrolysis workers, such data would be helpful in interpreting the significance of the lung cancers seen in the Finnish workers. A smoking prevalence in the Finnish workers similar to that observed in the Norwegian workers could readily explain the increased lung cancer rates seen in this study. This needs to be examined further.

Third, in the case of the nasal cancers, even though the Finnish workers may have been predominantly exposed to soluble nickel during their employment at the refinery, their previous job experiences as well as concomitant exposures to insoluble nickel compounds and acid mists make the establishment of a causal association with soluble nickel difficult. The very large nasal cancer risk in the Finnish workers is inconsistent with that found in other nickel refinery workers with a comparable (or higher) degree of soluble nickel exposure. It is notable that in up-dates of other cohorts, nickel-related nasal cancers have not been observed in workers first employed since around the mid-1950s. Adequate follow-up time exists for many of these workers. While it might be argued that the ability to detect such rare cancers in occupational workers is limited, if soluble nickel is really as potent a nasal cancer inducer as some would have the regulatory community believe, it is curious that only in the Finnish cohort have nasal cancers been detected in workers first employed since the mid-century. As these findings are inconsistent with all other studies on nickel workers, careful scrutiny must be given to these nasal cancers.

In short, there are many problems surrounding the Finnish studies and further information critical to their interpretation is required. A thorough discussion of these problems (and additional information) is provided in Appendix D.

2.6. OTHER OCCUPATIONAL EXPOSURE STUDIES

PAGES 3-6 THROUGH 3-7, SECTION 3.3:
First, data from the Wortley *et al.* (1992) and Horn-Ross *et al.* (1997) have been mixed-up in the NTP Draft RoC Background Document. A job matrix was used in the Wortley study, not the Horn-Ross study.

More importantly, it is highly questionable whether the studies by Wortley *et al.*, (1992) and Horn-Ross *et al.*, (1997) show any association of occupational exposure to nickel and cancer. In the case of Wortley *et al.*, the authors, themselves, note that potential exposure to chromium or nickel was not associated with significantly increased risk, nor could the exposures to the two metals be separated. Further, the fact that elevated laryngeal cancers have not been seen in other, much larger cohorts where workers have been involved in grinding operations (Arena *et al.*, 1998), suggests that the results seen in the Wortley *et al.* study may be due to chance or limitations in the design of the study (misclassification in job titles, multiple exposures, multiple statistical comparisons, *etc.*).

The Horn-Ross study is even more questionable as a useful source of information in that a self-reporting questionnaire was used to determine whether workers were "exposed" to nickel. Scanty information is provided on the questionnaire, and it is indeterminate whether the questionnaire was properly validated. The reader is only told that phrasing of questions was drawn from validated instruments "whenever possible." More importantly, although salivary cancer may be rare, elevated rates of it have never been reported in any other individual nickel cohorts studied, nor in the pooled analysis of cancer data in the ICNCM study. This lack of substantiating evidence from other nickel studies--some of which are very large (30,000-50,000+ workers)--renders the salivary cancers "found" in the Horn-Ross study particularly suspect. Either this study should not be reported, or its deficiencies and inconsistencies should be clearly noted.

2.7. EXPERIMENTAL CARCINOGENESIS

PAGE 4-1, SECTION 4.2.1, PARAGRAPH 3:
Evaluation of the adrenal tumorigenicity data from the NTP rat studies of inhalation exposure to nickel subsulfide and nickel sulfate hexahydrate demonstrated that at equivalent exposures of nickel in the nickel subsulfide-treated and nickel sulfate hexahydrate-treated animals, there was a completely different response with regard to the occurence of pheochromocytomas. Inhalation of 0.1 mg Ni/m^3 of nickel subsulfide caused an increase in this spontaneously occurring tumor while inhalation of 0.1 mg Ni/m^3 of nickel sulfate hexahydrate did not. This is a particularly important observation given the fact that the water soluble nickel sulfate hexahydrate would have caused higher blood Ni^{2+} levels than the poorly soluble nickel subsulfide. Higher blood Ni^{2+} would have resulted in higher Ni^{2+} levels in the adrenal medulla. The lack of adrenal tumors in the nickel sulfate hexahydrate treated animals **clearly suggests that the nickel ion is not responsible for the induction of these tumors.** Given that pheochromocytomas are spontaneously occurring endocrine tumors in the Fisher 344 rat, it is likely that the increase in these tumors over control levels seen in the nickel subsulfide and nickel oxide studies are related to secondary effects on endocrine homeostasis at the toxic doses of these compounds that were used in the studies.

It should also be noted that a similar response was observed in animals that inhaled talc (NTP, 1993). This response may be a particle effect-related response although it was not observed in animals that inhaled antimony trioxide or titanium dioxide. Ultimately, the significance of these tumors is unclear, but the NTP's own data show that they cannot have occurred as a direct effect of the nickel ion in the adrenal medulla!

PAGES 4-2 TO 4-4, SECTION 4.2.1-3:
It is disturbing that the discussion on animal carcinogenicity post IARC, would include just a cursory presentation of the data derived from the well-conducted animal bioassay in rats and mice by relevant

route of exposure (NTP, 1996), while focussing the discussion on the results of studies conducted by irrelevant routes of exposure, such as intraperitoneal, intrarenal or intramuscular, at one particular laboratory. Other post 1990 studies were ignored (*e.g.,* Muhle *et al.*, 1992).

PAGE 4-4, SECTION 4-2-3:
Even though the document focuses almost exclusively on the studies coming from one research group, the presentation and interpretation of the results from these studies is misleading and inaccurate. In the discussion of the Kazprzak *et al.* (1990) study, it should be noted that after 96 weeks the group of animals exposed solely to nickel acetate by intraperitoneal injection did not get excess tumors compared to saline controls (only one adenoma among 23 rats was found). Therefore, nickel acetate was not shown to be carcinogenic to rats by i.p. injection. A group of animals exposed solely to sodium barbital did get excess tumors (6 of 24 animals had adenomas, some had more than one) , indicating complete carcinogenic activity for this compound in the rat kidneys. This important control is not included in Table 4-1. In the presence of nickel acetate and sodium barbital more tumors were observed (13 of 24 animals had adenomas and 4 of them had carcinomas). These results are consistent with a possible "enhancing" role for soluble nickel in the kidney rather than an initiator/complete carcinogen role. These results are also in agreement with the results from the Kurokawa *et al.* (1985) study.

In the Diwan et al (1992) study, again intraperitoneal injection of nickel acetate by itself fails to induce kidney tumors in the offspring of treated female rats. These results confirm the lack of kidney carcinogenicity seen with nickel acetate alone by Kazprzak *et al.* (1990). Surprisingly, this study shows three-times as many pituitary tumors in offspring of nickel acetate treated rats (42%) than in offspring of sodium acetate ones (13%). It should be noted that the historical data for the Fischer 344 rat indicate an average of 23 percent and 45 percent pituitary adenoma incidence for males and females, respectively (Haseman *et al.*, 1990). The observed increases in pituitary tumors in offspring of animals treated with nickel acetate may be explained by a disruption of the endocrine system due to the toxic effects of the Ni^{2+} ion (quite evident in this study with 88% pup mortality) rather than to a carcinogenic effect. It has been shown that in the rat, pituitary tumors can occur as a consequence of hormonal disruption (Mennel, 1978). The lack of synergistic effects between sodium barbital and nickel acetate, as well as the lack of pituitary tumors in other studies (with soluble and insoluble nickel compounds) such as: transplacental study (Sunderman *et al.*, 1981), intraperitoneal study (Kasprzak *et al.*, 1990), oral studies (Ambrose *et al.*, 1976; Schoeder and Mitchener 1975), and inhalation NTP studies (NTP 1996) are consistent with this explanation. In addition no pituitary tumors have been detected in human epidemiologic studies.

The whole animal section should be rewritten with careful consideration of interpretation and conclusions derived from <u>all</u> the animal studies available in the nickel literature.

2.8. GENOTOXICITY

The section on genotoxicity and mechanism of carcinogenesis shows a very poor understanding of the significance and limitations of *in vitro* assays. In some cases, it appears that only the title of the articles cited were reviewed. Some examples of mistakes or omissions are listed below:

PAGE 5-3, PARAGRAPH 3:
It is stated that nickel sulfate induced transformation to anchorage-"*dependent*" instead of "*independent*" growth of primary human foreskin fibroblasts. This is not just a typo since it is repeated again on page 5-4 for nickel acetate.

PAGE 5-3, PARAGRAPH 3:
Regarding the work by Tveito *et al.* (1989), it is reported that "*human fetal kidney cortex explants did not become tumorigenic after 70-100 days of exposure to nickel sulfate.*" This statement is correct. However, some of the other important findings in this work were not reported while the same type of

findings in other studies were. For example, the fact that exposure to nickel sulfate resulted in immortalization and growth in soft agar (anchorage independence) was not reported.

PAGE 5-5, PARAGRAPH 4:
It is reported that "*...Ni^{2+} was effective in causing 8-OH-dG formation and double strand breaks in calf thymus DNA.*" What was not reported is that this effect was only seen when significant concentrations of *t*-butyl hydroperoxide and glutathione were also added to the reaction. Addition of 1 mM nickel chloride by itself did not cause any induction of oxidative damage.

A review of the genotoxicity data indicates that, in general, nickel compounds are not very genotoxic in standard *in vitro* assays. Nickel compounds have not been shown to induce gene locus mutations, but DNA strand breaks, chromosomal aberrations, and cell transformation have been consistently observed. It should be noted that although there are differences in the concentrations needed to see these effects, both soluble and insoluble nickel compounds can induce them. In general, much higher concentrations of soluble nickel compounds than of more insoluble nickel compounds are needed to see an effect. These results can be reconciled with the negative animal respiratory carcinogenicity data for soluble nickel compounds that have been discussed above. As mentioned before, ***it is the availability of nickel ions at nuclear sites within target cells that is important for carcinogenesis.*** *In vivo*, the clearance of soluble nickel compounds is so fast, nickel ion their transport into the cells is so inefficient (*i.e.*, nickel competes with mM levels of magnesium for transport via magnesium channels), and the affinity of nickel ions for proteins in the cytoplasm is so strong, that no accumulation of nickel ions at nuclear sites is expected to occur. When animals or humans are exposed to soluble nickel compounds by inhalation, the toxic response to soluble nickel compounds is evident before high enough concentrations of nickel ions can accumulate in the nucleus and cause heritable changes (see data from NTP 1996c report). *In vitro*, however, there is no clearance, and if high enough concentrations are added to the culture, the nuclear effects of nickel ions will also be observed with soluble nickel compounds.

A consideration of the interrelationship among clearance, toxicity, and availability of nickel ions at target sites needs to be taken into account when extrapolating *in vitro* results to the *in vivo* situation.

PAGE 5-5, PARAGRAPH 5:
Again, the data from another Kasprzak study (Kasprzak *et al.*, 1997) is incorrectly described. Male rats were injected with 90 µmoles (or 23 mg) nickel acetate tetrahydrate per kg body weight. The formation of oxidative damage in DNA isolated from liver or kidney tissue was examined as a function of time. Several oxidative lesions were found to be increased 1 day after injection. The increases in both tissues were very small (*e.g.,* 8-OH-dG went from 12.18 to 17.69 mol 8-OH-dG/10^5 mol of dG in kidney and from 10.20 to 14.95 mol 8-OH-dG/10^5 mol of dG, in liver). Some lesions persisted more in kidney (~15 mol 8-Oh-dG/10^5 mol of dG in treated versus 12.5 in controls) after 14 days, than in liver (~11 mol 8-OH-dG/10^5 mol of dG versus 10 in controls). The NTP Draft RoC Background Document considers that these results are an indication of the "*tissue-specific* [kidney] *response to Ni(II)-mediated oxidative DNA damage*", and that they are "*consistent with the kidney as primary target for Ni(II) carcinogenicity from soluble salts.*" These conclusions have to be seriously questioned based on the following facts:

- It cannot be concluded that nickel-induced oxidative damage is tissue specific to the kidney when only kidney and liver were looked at and when the increases at these two tissues were similar at 5.5 and 4.8 8-OH-dG/10^5 dG, respectively.

- Kidney is not the target organ for the carcinogenicity of certain nickel compounds. Animals and humans exposed by inhalation to certain nickel compounds experienced lung and/or nasal sinus tumors only. Animal exposed to nickel acetate by intraperitoneal injection did not get kidney tumors either. In addition, the NTP studies of nickel sulfate hexahydrate also showed no effects on the kidneys of exposed rats. This is particularly important since the nickel sulfate hexahydrate study would have had higher peak blood levels of nickel ion at equivalent doses than either the nickel oxide or nickel subsulfide studies. This is due to the dissolution (solubilization) rates of the different nickel

compounds in the respiratory tract (a fact which should have been made clear in Page 1-1, Paragraph 5 – see the corresponding comments for that section).

- Only male rats exposed to sodium barbital, with or without prior intraperitoneal injection of sodium acetate, got kidney tumors. Of the animal studies conducted with nickel subsulfide, only rats injected intrarenally got kidney tumors.

A reference is made in the NTP Draft RoC Background Document to another study by the Kasprzak group (Higinbotham *et al.*, 1992). In this study 12 renal tumors induced by intrarenal injection with nickel subsulfide were analyzed for mutations at the K-ras oncogene. Only one tumor showed a mutation in codon 12 of the K-ras gene. This mutation was a G to T transversion. According to the NTP Draft RoC Background Document this mutation is the result of a 8-OH-dG lesion induced by nickel. This conclusion seems premature at best, given that it is not known with certainty whether the observed oncogene mutations seen in some tumors (ras, p53) are directly related to the treatment that induced that tumors or simply the result of selection by the treatment of preexisting mutations. Analysis of mutations at the p53 gene of the nickel subsulfide-induced tumors mentioned above failed to reveal any changes (Weghorst *et al.*, 1994). A further study of the human kidney cells immortalized *in vitro* by exposure to nickel sulfate (Tveito *et al.*, 1989) revealed clones with T to C transition mutations (rather than G to T transversions) in the p53 gene (Maehle *et al.*, 1992 What does it all mean? At present, the significance of these findings remains to be determined.

2.9. OTHER RELEVANT DATA

PAGE 6-2, SECTION 6.2, PARAGRAPH 3:
The information in the last paragraph of this section comes from a study by Sunderman and co-workers (1989). This study is cited but not referenced. The importance of fasting is critical to the results and understanding of this study. Intestinal absorption of nickel will largely depend upon the presence of food already in the stomach and the type of food ingested (Solomons *et al.*, 1982; Foulkes and McMullen, 1986). It should be clarified in the text that the maximum absorption of nickel from an oral dose of nickel sulfate (25%) was only observed in volunteers that fasted overnight before drinking water. Under more common intake conditions about 5% of nickel will be absorbed orally.

PAGE 6-2, SECTION 6.3, PARAGRAPH 4:
The NTP Draft RoC Background Document states that *"In blood and urine, soluble nickel compounds and nickel metal powder are more easily measured than less soluble nickel compounds (Sunderman et al., 1986)."* This statement is incorrect. The only thing that can be found and measured in blood and urine is the Ni^{2+} ion. What the Sunderman paper concluded was that monitoring Ni^{2+} levels in blood or urine could be useful as an indication of exposure to soluble nickel compounds or very finely divided nickel metal powder. However, these parameters would not be useful to evaluate inhalation exposure to less soluble nickel compounds due to the slow lung clearance of these compounds.

2.10. MECHANISMS OF CARCINOGENESIS

PAGE 7-1, SECTION 7.0, PARAGRAPHS 1-2:
The first two paragraphs in this section indicate that the fact that high concentrations of soluble nickel compounds can, in some studies, induce DNA damage *in vitro* and *in vivo* (after injection), is a demonstration that the ionic nickel may be the carcinogenic agent. A more appropriate conclusion would be that because soluble and insoluble nickel compounds can produce some level of DNA damage *in vitro* and *in vivo*, the nickel ions present at cellular nuclear sites appear to have the potential to cause adverse genotoxic effects. Whether this genotoxic potential will translate or not into carcinogenic potential will depend on many other factors (such as route of exposure, particle size, solubility of the compound, clearance, *etc.*) that will ultimately determine the availability of nickel ions at nuclear sites within target

cells. Animal and human data needs to be used to ultimately determine the carcinogenic potential of the individual nickel compounds.

PAGE 7-1, PARAGRAPH 3:
The animal data for soluble compounds are indeed reviewed on paragraph 3. However, only the Kasprzak studies by intraperitoneal route are mentioned excluding the other dozen negative studies, by relevant routes of exposure and in multiple animal species. The significance of the intraperitoneal studies is further obfuscated by failing to mention the very unique conditions under which rats developed renal tumors (males only, in the presence sodium barbital exposure only) and the high toxicity experienced by the pups with pituitary tumors in the transplacental carcinogenicity study.

It is surprising that again, the NTP inhalation studies (1996) with nickel subsulfide, high temperature (green) nickel oxide and nickel sulfate hexahydrate in two animal species were not mentioned at all.

PAGE 7-1, PARAGRAPH 4:
The next few paragraphs in the NTP Draft RoC Background Document address a possible mechanism for nickel-induced carcinogenesis. It is apparent that the authors of this section of the NTP Draft RoC Background Document do not quite understand some of the issues pertaining to respiratory tract physiology. Two different issues are of concern for inhalation exposure to nickel compounds: toxicity and carcinogenicity. With regard to toxicity, damage to lung cells appears to occur by the action of nickel ions at the cell surface or in the cytoplasm, due to the great affinity of nickel ions for proteins. In this regard, soluble nickel compounds are toxic to the lungs of animals at lower concentrations than insoluble nickel compounds are. Toxicity for particulate nickel compounds appears to be related to their solubility in biological fluids[4].

With regard to carcinogenicity, the target cells for tumors in the lung are epithelial cells. The lungs contain a mucociliary escalator that moves particles up towards the throat for elimination by oral route. They also contain alveolar macrophages. It is the function of the macrophages to phagocytize foreign particles and bacteria that get deposited deep into the lung. Macrophages posses a very specialized way of engulfing particles and disposing of them by dissolving them under acidic pH in the phagosomes or carrying them via the lymphatic system for elimination. They also have a great capacity to generate oxidative damage. If the above mentioned mechanisms fail to completely eliminate all the particles (high exposure), lung epithelial cells themselves may come in contact with particles. All cells, including lung epithelial cells, have the ability to endocytize particles to varying degrees. If the epithelial cells endocytize nickel-containing particles (insoluble), nickel ions may be released inside the acidic endocytic vesicles. Furthermore, these vesicles appear to fuse with the nuclear membrane allowing a high pulsatile delivery of nickel ions to nuclear chromatin. Once in the nucleus, nickel ions can replace magnesium binding to histones in the chromatin. It is not clear what the exact changes caused by nickel ions are that can result in tumor induction (*e.g.,* changes in chromatin condensation, oxidative damage, *etc.*). In this case, the bioavailability of nickel ions at nuclear sites within the epithelial cells will be greater for particulate compounds of intermediate solubility (*e.g.,* nickel subsulfide). In contrast, soluble nickel compounds are rapidly eliminated by dissolution into the blood and excretion through the kidney. Soluble nickel compounds cannot be endocytized by epithelial cells (*i.e.,* they quickly dissociate to free ions hence, there are no particles to endocytize). Therefore, the only way in which soluble nickel compounds could cause a high accumulation of nickel ions in the nucleus of epithelial cells is by being present in the lung at such high concentrations that even with rapid clearance they can compete with magnesium (mM) levels for uptake into the cytoplasm. They also have to be able to concentrate in the cytoplasm at high enough levels to reach the nucleus inspite of their high binding affinity for cytoplasmic proteins. *In vivo,* this will not be achieved due to the high toxicity of nickel ions that will be manifested before such high inter- and intracellular concentrations of nickel ions can be achieved.

[4] For low-solubility particles, toxicity and carcinogenicity may occur at high concentrations through a secondary mechanism related to impaired clearance.

The NTP Draft RoC Background Document continues to confuse the concepts of toxicity and carcinogenicity as shown in the following examples:

PAGE 7-2, PARAGRAPH 1:
It is mentioned here that *"Tumor induction was thought to be related to ...or by the ability of the cell to incorporate the compound (e.g., phagocytosis). However, Kasprzak and Ward (1991) found that stimulated phagocytes, rather than enhancing carcinogenic response, actually strongly inhibited muscle tumor development in rats injected with nickel subsulfide."* It is expected that stimulated phagocytosis by macrophages will have the opposite effect on tumor induction (by decreasing particle availability to target cells) than stimulated endocytosis by target cells would (by increasing nickel ion availability at nuclear sites). By contrast, the language in the NTP Draft RoC Background Document suggests that all processes of phagocytosis by macrophages or endocytosis by target cells are irrelevant for tumor induction.

PAGE 7-2, PARAGRAPH 2:
It is mentioned here that *"...particles dissolved in the acidic pH of cytoplasm."* This is incorrect, cytoplasm is not acidic, phagosomes or endocytic vesicles are.

The last two sentences of this paragraph were obviously intended by the NTP Draft RoC Background Document authors to support each other. Unfortunately, although each statement is correct by itself, they do not relate to each other in the context of this document. The authors have confused phagocytosis by macrophages, a protective mechanism that reduces the toxicity/carcinogenic potential of insoluble nickel compound particles, and endocytosis by respiratory epithelial cells which precipitates tumor formation. Enhancing macrophage phagocytosis will enhance clearence of particles reducing the chance that epithelial cells will endocytize particles and become transformed.

PAGE 7-3, PARAGRAPH 2:
The first sentence in this paragraph is wrong. Soluble nickel salts are not less toxic than insoluble nickel compounds in animal models. The NTP Draft RoC Background Document appears to be misquoting the following statement included in Costa (1991) and cited in Costa 1995: *"Water soluble nickel salts are generally less carcinogenic in experimental animals because they do not get taken up to a degree similar to that for the particulate nickel compounds that yield high concentrations of nickel inside cells."*

PAGE 7-3, PARAGRAPH 2:
In this paragraph, the Kasprzak intraperitoneal studies are cited again as compelling evidence for the carcinogenicity of soluble nickel compounds, the NTP inhalation studies are left out again, and this time the whole discussion is wrapped up with the categorical statement that *"...macrophage solubilization is not required for carcinogenesis to occur with nickel."* This is the last of the many examples in this NTP Draft RoC Background Document showing a lack of understanding of the mechanistic data (see comments for Page 7-2, Paragraphs 1 and 2).

PAGE 7-4, PARAGRAPH 2:
Surprisingly, the last sentence in the document makes a reasonable assessment even if it is ignored for the purposes of nickel compounds carcinogenic classification:

"Overall, it appears that the ionic form of nickel is the ultimate carcinogenic species, and biokinetic factors may dictate the carcinogenic potential of the various soluble and insoluble nickel compounds."

3. Conclusion

NiPERA's major objection to the NTP's proposal to list *Nickel and Nickel Compounds* as *"known human carcinogens"* in the Ninth Biennial Report on Carcinogens is that it fails to recognize differences in the carcinogenic potential of the various forms of nickel. Each compound or species of a metal, like nickel, has its own physico-chemical properties that dictate how it behaves under a given set of conditions,

including interactions with biological organisms. Thus, the fact that one form of nickel may be carcinogenic via a particular route of exposure (*e.g.*, nickel subsulfide by inhalation) does not mean that a second nickel species will be carcinogenic as well or that the first nickel species will be carcinogenic via a different route of exposure (*e.g.*, ingestion). For nickel and its compounds, this observation holds true even if the free metal ion is assumed to be the active carcinogenic agent, because the different physico-chemical properties of various forms of the metal will largely determine the extent to which the free metal ion can be made bioavailable and delivered to a relevant biological site (*e.g.*, the nucleus of a lung epithelial cell).

Examination of the *in vitro*, animal, and epidemiologic data pertaining to commercially relevant nickel compounds[5] confirms that these compounds have very different biological behaviors, particularly with regard to respiratory carcinogenicity. Nickel subsulfide is likely to be carcinogenic to humans. Soluble nickel compounds, by themselves, have not been demonstrated to be carcinogenic to humans, although an enhancing (promoter) effect on other carcinogens is possible. High concentrations of oxidic nickel mixtures (*i.e.*, Ni-Cu oxides mixed with low-temperature [black] and high-temperature [green] NiO) appear to be carcinogenic in epidemiologic studies of nickel refinery workers. Exposures to nickel silicates-oxides and complex nickel oxides devoid of copper have not resulted in excess cancer risks in other human cohorts. Exposure to metallic nickel particles in the workplace does not appear to pose a respiratory carcinogenic risk for humans. Finally, nickel carbonyl is so acutely toxic that it is used in closed systems and humans are typically exposed only in accident scenarios. The high acute toxicity of nickel carbonyl has limited its examination for carcinogenic effects. The human and animal data on the potential carcinogenicity of nickel carbonyl are scant and only non-standard animals studies with exposures above the Maximum Tolerated Dose (MTD) have yielded evidence of a carcinogenic effect.

Against this background, NiPERA believes that the NTP proposal to sweep metallic nickel and all nickel compounds into the single category of "*known human carcinogens*" is inconsistent with both the epidemiological and toxicological data and is at odds with the best current understanding of the likely mechanism of nickel-related carcinogenicity.

[5] The classes of commercially relevant nickel compounds are: metallic nickel, oxidic nickel (including nickel oxides, hydroxides, silicates, carbonates, and complex nickel oxides), sulfidic nickel (including nickel sulfide and subsulfide), water soluble nickel compounds (including hydrated forms of nickel acetate, sulfate, chloride, *etc.*), and nickel carbonyl. Metallic, oxidic, and sulfidic nickel compounds and nickel carbonyl are insoluble in water.

4. References Cited in these Comments that are not Included in the NTP Draft RoC Background Document

Ambrose, A. M., Larson, P. S., Borzelleca, J. F., and Hennigar, G. R. Jr. (1976). Long term toxicologic assessment of nickel in rats and dogs. *J. Food Sci. Technol.*, 13, 181-187.

Arena, V. C.; Sussman, N. B.; Redmond, C. K.; Costantino, J. P. and Trauth, J. M. (1998). Using alternative comparison populations to assess occupation-related mortality risk. *Journal of Occupational and Environmental Medicine*, 40, 907-916.

Burges, D. C. L. (1980). Mortality study of nickel platers. In: Brown, S. S. and Sunderman, F. W., Jr., eds. Nickel toxicology: Proceedings of the 2nd international conference, September, Swansee, Wales. London, United Kingdom: Academic Press, pp. 15-18.

Foulkes, E. C. and McMullen, D. M. (1986). On the mechanism of nickel absorption in the rat jejunum. *Toxicology*, 38, 5-42.

Gilman, J. P. W. (1962). Metal Carcinogensis. II. A study of the carcinogenic activity of cobalt, copper, iron and nickel compounds. *Cancer Res.*, 22, 158-162.

Haseman, J. K.; Eustis, S. L.; and Arnold, J. (1990). Tumor Incidences in Fischer 344 Rats: NTP Historical Data. In: *Pathology of the Fischer Rat: Reference and Atlas*, edited by Boorman, G.A.; Eustis, S.L.; Elwell, M.R.; Montgomery, Jr., C.A.; and MacKenzie, W.F., pp. 555-564, Academic Press, San Diego, California.

IARC (1992). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Occupational Exposures to Mists and Vapors from Strong Inorganic Acids and Other Industrial Chemicals; v. 54. Geneva, Switzerland: World Health Organization.

Kasprzak, K. S., Gabryel, P., and Jarczewska, K. (1983). Carcinogenicity of nickel (II) hydroxides and nickel(II) sulfate in Wistar rats and its relation to the *in vitro* dissolution rates. *Carcinogenesis* 4, 275-279.

Kurokawa, Y.; Matsushima, M.; Imazawa, T.; Takamura, N.; Takahashi, M. (1985). Promoting effect of metal compounds on rat renal tumorigenesis. *J. Am. Coll. Toxicol.* 4, 321-330.

Maehle, L.; Metcalf, R. A.; Ryberg, D.; Bennett, W. P.; Harris, C. C.; and Haugen, A. (1992). Altered p53 gene structure and expression in human epithelial cells after exposure to nickel. *Cancer Res.*, 52, 218-221

Mennel, H.D. (1978). Transplantation of tumors of the nervous system induced by resorptive carcinogens. *Neurosurg. Rev.*, 1, 123.

Moulin, J. J., Mantout, B., Portefaix, P., Wild, P., Fournier-Betz, M., Mur, J. M. , and Smagghe, G. (1992). Etude épidémiologique de mortalité dans deux aciéries d'acier inoxydable. [Historical prospective mortality study in two stainless steel factories]. *Arch. Mal. Prof. Med. Trav. Secur. Soc.*, 53, 157-166.

Muhle, H., Bellman, B., Takenaka, S., Fuhst, R., Mohr, U., and Pott, F. (1992). Chronic effects of intratracheally instilled nickel-containing particles in hamsters. In: Nickel and Human Health: Current Perspectives. Nieboer, E.; Nriagu, N. O., eds. New York, NY: John Wiley & Sons, Inc. p. 467-479.

NTP (National Toxicology Program) Technical Report. (1993). Toxicology and carcinogenesis studies of talc (CAS No. 14807-96-6) in F344/N rats and B6C3F$_1$ mice (inhalation studies). NIH publication No. 93-3152.

Pang, D., Burges, D. C., and Sorahan, T. (1996). Mortality study of nickel platers with special reference to cancers of the stomach and lung, 1945-93. *Occup. Environ. Med.*, 53, 714-717.

Payne, W. W. (1964). Carcinogenicity of nickel compounds on experimental animals. *Proc. Am. Assoc. Cancer Res.* 5, 50.

Schroeder, H. A., Balassa, J. J., and Vinton, W. H. (1964). Chromium, lead, cadmium, nickel and titanium in mice: effect on mortality, tumors and tissue levels. *J. Nutr.*, 83, 239-250.

Schroeder, H.A., Mitchener, M., and Nason, A.P. (1974). Life-term effects of nickel in rats: survival, tumors, interactions with trace elements and tissue levels. *J. Nutr.*, 104, 239-243.

Schroeder, H. A., and Mitchener, M. (1975). Life-term effects of mercury, methyl mercury, and nine other trace metals on mice. *J. Nutr.*, 105, 452-458.

Solomons, N. W., Viteri, F., Shuler, T. R., and Nielsen, F. H. (1982). Bioavailability of nickel in man: Effects of foods and chemically-defined dietary constituents on the absorption of inorganic nickel. *J. Nutr.,* 112, 39-50.

Steenland, K.; Schnorr, T.; Beaumont, J.; Halperin, W.; and Bloom, T. (1996) Incidence of laryngeal cancer and exposure to acid mists. *Br. J. Ind. Med.,* 45, 766-776.

Sunderman, F. W.; McCully, K. S.; and Rinehimer, L. A. (1981). Negative test for transplacental carcinogenicity of nickel subsulfide in Fischer rats. *Res. Commun. Chem. Pathol. Pharmacol.* 31, 545-554.

Sunderman, F . W., Jr., Hopfer, S. M., Swenney, K. R., Marcus, A. H., Most, B. M., and Creason, J. (1989). Nickel absorption and kinetics in human volunteers. *Proc. Soc. Exp. Biol. Med.,* 191, 5-11.

Verma, D. K.; Julian, J. A.; Roberts, R. S.; Muir, D. C. F.; Jadon, N.; and Shaw, D. S. (1992) Polycyclic aromatic hydrocarbons (PAHs): A possible cause of lung cancer mortality among nickel/copper smelter and refinery workers. *Am. Ind. Hyg. Assoc. J.,* 53, 317-324.

Weghorst, C. M.; Dragnev, K. H.; Buzard, G. S.; Thorne, K. L.; Vandeborne, G. F.; Vincent, K. A.; and Rice, J. M. (1994). Low incidence of point mutations detected in the p53 tumor suppressor gene from chemically induced rat renal mesenchymal tumors. *Cancer Res.,* 54, 215-219.

APPENDIX A

REVIEW OF THE STUDY BY DIWAN *ET AL.* TITLED, *TRANSPLACENTAL CARCINOGENIC EFFECTS OF NICKEL(II) ACETATE IN THE RENAL CORTEX, RENAL PELVIS AND ADENOHYPOPHYSIS IN F344/NCR RATS.*

An examination of the potential for nickel to induce transplacental carcinogenesis was conducted by Diwan and co-workers (1992) at the U.S. National Cancer Institute. In that study, the soluble compound, nickel acetate (NiAct), was administered to pregnant Fischer 344 rats by intraperitoneal injection during the last third of their gestation period. Three treatment groups were utilized; the first exposed to 90 µmol/kg of nickel acetate dissolved in distilled water on gestation day 17 (the day mating was confirmed was designated gestation day 1); the second exposed to 45 µmol/kg of nickel acetate on each of days 16 and 18 of gestation; and the third also exposed to 45 µmol/kg of nickel acetate, but on each of gestation days 12, 14, 16, and 18. Control animals were exposed to 180 µmol/kg of sodium acetate on gestation day 18. The animals were allowed to deliver and nurse their young. After weaning, the pups were randomly divided into two subgroups (A and B) within each prenatal exposure group. The A subgroups were maintained on-study with no further treatment while the B subgroups were administered the tumor promoter, sodium barbital (NaBB), in their drinking water at a 4 percent concentration from the fourth week postpartum until the study end at 85 weeks postpartum. All the pups on this study were necropsied for histopathological assessment upon their death or at the study termination.

The authors reported that the four day exposure regimen used for the animals in group three resulted in 100 percent mortality. In examining the offspring in groups one and two they stated that nickel acetate was a complete carcinogen for the induction of pituitary tumors seen in the A subgroups which were not exposed to the tumor promoter NaBB. In addition, sodium barbital (NaBB)-promoted renal tumors were observed in both male adult rats administered nickel acetate (an earlier study by Kasprzak *et al.* employing a single i.p. injection of 90 µmol NiAct/kg bw) and the male offspring of dams administered NiAct (either single i.p. injection, 90 µmol NiAct/kg on day 17 of gestation or two i.p. injections, 45 µmol NiAct/kg each, on days 16 and 18 of gestation). Animals administered NiAct but not NaBB did not develop renal tumors. The authors concluded that nickel acetate initiated the formation of renal cortical and pelvis (medullary) tumors in the two surviving B subgroups exposed to the tumor promoter NaBB during their lifetimes.

Study Critique

A number of considerations in the design, conduct and interpretation of this study cast doubt on the validity of the conclusion reached by Diwan and co-workers. These considerations are discussed as follows:

Sodium Barbital Carcinogenicity

Subsequent work by these authors (Kurata *et al.*, 1993) has demonstrated that sodium barbital by itself is a nongenotoxic nephrotoxicant carcinogen and an inducer of neoplastic tubular lesions in the Fisher 344/NCr rat. The fact that NaBB by itself acted as a complete kidney carcinogen (initiation and promotion) while NiAct by itself did not, can certainly not be used as evidence for the carcinogenicity of soluble nickel compounds.

Diwan *et al.* (1992) concluded that NiAct was behaving as a tumor initiator in their study. An alternative explanation for the increased presence of tumors in NaBB-exposed animals whose mothers received NiAct is possible and more in agreement with the rest of the animal and human data on soluble nickel compounds. The presence of Ni ions during kidney development in the fetus could have caused toxicity and increased cell proliferation leading to a greater fixation of spontaneous lesions. The increased number of lesions at birth would not, by themseleves, resulted in tumors unless they were promoted further in males by NaBB. This explanation is consistent with a promoter rather than an initiating role for soluble nickel compounds.

Design Considerations

Review of this study revealed a number of design flaws. Specifically, the study was designed with a small number of F_1 animals (approximately 60/group; 30/subgroup A and B) which were assessed for transplacental carcinogenicity. The authors do not say how many dams were actually treated in this study, but the Fischer 344 rat has a litter size of approximately nine pups indicating that either there was a large incidence of postnatal mortality (*i.e.* offspring dead at birth) or the number of litters that were treated with

nickel acetate was approximately 8/group. The data from this study were erroneously analyzed on the basis of the pup as the unit of statistical significance. The authors stated that they had an *n* of approximately 30/subgroup. The smallest "unit" that can be individually treated in a study such as this is the litter; therefore, the actual *n* for this study is the number of maternal animals treated in each group (*i.e.* approximately 8/group). This design flaw is further exacerbated by an inappropriate selection of statistical methods for the analysis of some of the data generated in this study. Specifically, t-test's were used to analyze data from multiple groups. This approach increases the possibility of obtaining a false positive result.

Tumorigenicity Issues

The results of this study indicated an increase in renal and pituitary tumors in the offspring exposed to nickel while in utero. Renal tumors in rats have been associated with a gender dependent susceptibility pattern which has been observed with a variety of renal carcinogens, including unleaded gasoline (Montgomery and Seely, 1990). This syndrome, known as α2μ-globulin (α2μ-g) nephropathy, has been associated with dose-related increases in renal adenomas and adenocarcinomas in male Fischer 344 rats (the rats used in this study). The EPA, after considerable study and panel review, issued a science policy that states: " Male rat renal tubule tumors arising as a result of a process involving α2μ-g accumulation do not contribute to the qualitative weight-of-evidence that a chemical poses a human carcinogenic hazard. Such tumors are not included in dose-response extrapolations for the estimation of human carcinogenic risk" (U.S. EPA, 1991).

In this study, renal tumors were seen only in male offspring of exposed maternal animals. Diwan and co-workers stated that they did not think this syndrome was operating in their study because no lesions or hyaline droplets compatible with α2μ-g nephropathy were found in the kidneys in male offspring of either the subgroup A (NiAct alone) or subgroup B (NiAct + NaBB) animals " in spite of careful scrutiny of histologic sections." However, Montgomery and Seely (1990) state that " ....although the lesions associated with short-term exposure to chemicals causing increased α2μ-g in the kidney may be marked and characteristic of hyaline droplet nephropathy, in some studies there are minimal changes on routine hematoxylin and eosin stained sections." They note that other staining methods must be used to detect the α2μ-g lesions. Diwan and co-workers used only a routine hematoxylin and eosin staining technique. Without a definitive assessment of the potential role of α2μ-g in this study, no conclusions based on the renal tumor incidence in the study should be made.

In evaluating the pituitary tumor data in this study, Diwan and co-workers concluded that nickel acetate is a complete carcinogen since it did not require the presence of a promoter (NaBB) to cause a significant increase in tumorigenicity. The tumorigenicity data for the pituitary were analyzed by the authors based on the total tumor incidence. No differentiation between adenomas and carcinomas was made in the analysis although, the incidence of each type of tumor was reported. Adenoma incidence ranged from 7 to 29 percent in the control groups and from 19 to 29 percent in the treated groups. Historical data for the Fischer 344 rat indicate an average of 23 percent and 45 percent adenoma incidence for males and females, respectively (Haseman *et al.*, 1990). Therefore, the data from this study do not support the assertion of an effect on pituitary adenoma incidence. The incidence of pituitary carcinoma in this study ranged from 7 to 31 percent in the treated subgroups, and was nonexistent in the control subgroups.

To ascertain the significance of the pituitary tumor findings in this study it should be considered that pituitary tumors can occur as a consequence of hormonal disruption in the rat (Mennel, 1978). This mechanism has not been shown to have a corollary in humans and therefore, may not be relevant for risk assessment purposes. It is possible therefore, that toxicity of nickel could disrupt endocrine homeostasis producing the indirect effect of inducing hormonal disruption in the rat which could lead to pituitary tumors. The toxic effects of the Ni^{2+} ion were quite evident in this study and resulted in 88% pup mortality. The lack of synergistic effects between sodium barbital and nickel acetate, as well as the lack of pituitary tumors in other studies (with soluble and insoluble nickel compounds) such as: a transplacental study by Sunderman *et al.* (1981), an intraperitoneal study by Kasprzak *et al.* (1990), oral studies by Ambrose *et al.* (1976) and by Schoeder and Mitchener (1975), and the inhalation studies by the NTP (1996) are consistent with this explanation. In addition no pituitary tumors have been detected in human epidemiologic studies.

Relevance of Route of Exposure

Many of these studies utilize a route of exposure chosen to maximize effect rather than reproduce human exposure patterns. The relevance of the route of exposure is exemplified by Diwan and co-worker's statement that "*the realistic routes of exposure to [nickel] are air, drinking water, and food*" (Diwan *et al.*, 1992). In view of this fact, one of Diwan's co-authors conducted a study to test the transplacental carcinogenic potential of NiAct *via* the route of oral exposure. This study showed no increased incidence of tumors in the kidney of exposed rats (Kasprzak, 1995).

Further evidence of the inadequacy of the routes of exposure used in the studies by Diwan and co-workers can be found in the Environmental Protection Agency's 1991 revised drinking water document. The Agency states that "*[M]any nickel compounds cause tumors via intraperitoneal (i.p.) or intrarenal injection. In general, these studies have found tumors only at the site of injection, although a few distant site responses were also seen. However, injection studies are not particularly relevant to human exposure.*" The Agency further notes that "*[A]s expected from the low gastrointestinal absorption, the toxicity of nickel in animal studies is much lower by the oral route than by parenteral routes. In the excretion study by Ho and Furst (1973) no overt toxic effects were observed in anesthetized rats given an oral dose of ≤ 64 mg Ni/kg body weight in the form of nickel chloride. The same dose given i.p. resulted in the death of 60 % of the animals.*" The LD_{50} for NiAct given i.p. is 8 mg Ni/kg body weight whereas the LD_{50} for NiAct given orally is 116-120 mg Ni/kg body weight (Haro *et al.*, 1968).

Inconsistencies With Other Studies

Neither the NTP inhalation study with rats and mice (NTP 1996) or several oral studies in various animal species (Schroeder *et al.*, 1974, 1964; Schroeder and Mitchener, 1975; Ambrose *et al.*, 1976) provide evidence of carcinogenicity for soluble nickel compounds. In the recent NTP inhalation study, about 100 rats and 100 mice were exposed for two years to near MTD concentrations of nickel sulfate hexahydrate and not tumors were observed at any site. The Ambrose study (1976) is particularly important because pathology was done on both adult rats in the 2-year feeding study *and* the F_{3b} offspring (10 males and females each) of the reproductive study (a fact often overlooked when this study is evaluated.) Renal tissues were examined. No nickel-related tumors were found in the adult rats and no nickel-related kidney lesions were found in the offspring. This study, therefore, suggests that nickel sulfate ($NiSO_4$), a more soluble compound than NiAct, did not behave as a complete carcinogen (in the case of the 2-year study) and did not result in kidney lesions in the offspring exposed *in utero*. The relevance of this work to the Diwan study (1992) concerns the pituitary tumors observed in that study. The renal tumors seen in that study only developed when promoted, but the pituitary tumors ostensibly were the result of a complete carcinogenic effect of NiAct which was not replicated in the Ambrose study (1976).

Formation of DNA Adducts

In search of the mechanism of the carcinogenesis evidenced in NiAct + NaBB treated rats in Diwan's study (1992), co-workers Kasprzak and Mishra have published a series of studies detailing the formation of DNA adducts formed in the kidneys of male and female adult rats as well as their offspring. The authors theorized that 8-hydroxy deoxyguanosine (8-OH-dG) DNA adducts might be the initial damage that lead to the renal carcinogenicity. This purine adduct was found to be elevated in the kidneys of adult male rats administered NiAct (Kasprzak *et al.*, 1992). Likewise, adult male rats administered NiAct + NaBB developed tumors (Kasprzak *et al.*, 1990). The offspring (males and females combined) of dams administered NiAct had elevated 8-OH-dG levels and, likewise, the male offspring administered NiAct + NaBB developed kidney tumors (Diwan *et al.*, 1992). As further "evidence" of the 8-OH-dG/tumor induction theory, the authors noted that 8-OH-dG was not elevated in the liver and, likewise, no liver tumors developed.

The authors of these papers failed to consider the *in vitro* literature in formulating their 8-OH-dG theory. Since 8-OH-dG adducts affect single DNA base pairs the heritable mutagenic outcome of the adduct would be a point mutation. The *in vitro* literature has demonstrated conclusively that nickel does not cause point mutations. Therefore, 8-OH-dG adducts cannot be the mechanism by which renal tumors are caused in the

Diwan/Kasprzak/Mishra series of studies. In fact, the concentration mechanisms of renal excretion indicate that high levels of nickel ion are probably produced within the nephrons of the kidney. It is not surprising therefore, that 8-OH-dG adducts are seen in renal DNA, but *in vitro* research has also demonstrated that there are ample repair mechanisms for such damage in the kidney. In addition, it has been demonstrated that oxygen radicals are needed to induce point mutations with nickel. Since the kidney has extremely high concentrations of the oxygen radical quenching proteins glutathione and metallothionine, it is unlikely that the 8-OH-dG adducts seen in these studies have any bearing on the induction of the renal tumors seen in rats.

In the Kasprzak 1992 paper on DNA base damage, the authors mentioned that marked sex differences in the susceptibility of rats to renal carcinogenesis have been seen and they, therefore, stated that the possible significance of 8-OH-dG formation in renal DNA " must be viewed with caution." Since it is clear that mechanism of tumor induction is extremely unlikely to involve 8-OH-dG adducts, the role of $\alpha 2\mu$-globulin in inducing these tumors is still the only theory that accounts for the male specific pattern of tumorigenicity seen in the Diwan/Kasprzak/Mishra studies.

References

Ambrose, A.M.; Larson, P.S.; Borzelleca, J.F.; and Hennigar, G.R., Jr. Long term toxicologic assessment of nickel in rats and dogs. *J. Food Sci. Technol.* 13: 181-187 (1976).

Creason, J.P.; Svendsgaard, D.; Bumgarner, J.; Pinkerton, C. and Hinners, T. Maternal-fetal tissue levels of 16 trace elements in 8 selected continental United States communities. In: *Trace Substances in Environmental Health--X,* edited by Hemphill, D.D., pp. 53-62 (1976).

Diwan, B.A., Kasprzak, K.S., and Rice, J.M. Transplacental carcinogenic effects of nickel(II) acetate in the renal cortex, renal pelvis and adenohypophysis in F344/NCr rats. Carcinogenesis 13(8): 1351-1357 (1992).

EPA. *Alpha 2μ-Globulin: Association With Chemically Induced Renal Toxicity and Neoplasia in the Rat.* EPA/625/3-91/019F, U.S. Environmental Protection Agency, Washington, D.C. (1991).

Haro, R.T.; Furst, A.; and Falk, H.L. Studies on the acute toxicity of nickelocene. *Proc. West. Pharmacol. Soc.* 11:39-42 (1968).

Haseman, J.K.; Eustis, S.L.; and Arnold, J. Tumor Incidences in Fischer 344 Rats: NTP Historical Data. In: *Pathology of the Fischer Rat: Reference and Atlas*, edited by Boorman, G.A.; Eustis, S.L.; Elwell, M.R.; Montgomery, Jr., C.A.; and MacKenzie, W.F., pp. 555-564, Academic Press, San Diego, California (1990).

Ho, W. and Furst, A. Nickel excretion by rats following a single treatment. *Proc. West. Pharmacol. Soc.* 16: 245-248 (1973).

Kasprzak, K. S. personal communication with NiPERA. (1995).

Kasprzak, K. S.; Diwan, B. A.; Konishi, N.; Misra, M.; Rice, J. M. Initiation by nickel acetate and promotion by sodium barbital of renal cortical epithelial tumors in male F344 rats. *Carcinogenesis* 11: 647-652 (1990).

Kasprzak, K. S.; Diwan, B. A.; Rice, J. M.; Misra, M.; Riggs, C. W.; Olinski, R.; Dizdaroglu, M. Nickel(II)mediated oxidative DNA base damage in renal and hepatic chromatin of pregnant rats and their fetuses. Possible relevance to carcinogenesis. *Chem. Res. Toxicol.* 5: 809-815 (1992).

Kuehn, K. and Sunderman, F.W. Dissolution half-times of nickel compounds in water, rat serum, and renal cytosol. *J. Inorg. Biochem.* 17: 29-39 (1982).

KurataY.; Diwan, B. A.; Uno, H.; Rice, J. M.; and Ward, J. M. pathology of preneoplastic and neoplastic renal tubular lesions induced in F-344 rats by sodium barbital, a nongenotoxic renal carcinogen and nephrotoxicant. *Toxicol. Pathol.*, 21(1):35-45 (1993).

Mennel, H.D. Transplantation of tumors of the nervous system induced by resorptive carcinogens. *Neurosurg. Rev.*, 1: 123 (1978).

Metzler, M. Mechanisms of Carcinogenesis Induced by Diethylstilbestrol. In: *Comparative Perinatal Carcinogenesis*, edited by Schuller, H.M., pp. 137-150, CRC Press, Boca Raton, Florida (1984).

Misra, M.; Olinski, R.; Dizdaroglu, M.; Kasprzak, K.S. Enhancement by L-histidine of nickel(II)-induced DNA-protein cross-linking and oxidative DNA base damage in the rat kidney. *Chem. Res. Toxicol.* 6: 33-37 (1993).

Montgomery, Jr., C.A. and Seely, J.C. Kidney. In: *Pathology of the Fischer Rat: Reference and Atlas*, edited by Boorman, G.A.; Eustis, S.L.; Elwell, M.R.; Montgomery, Jr., C.A.; and MacKenzie, W.F., pp. 127-154, Academic Press, San Diego, California (1990).

NTP (National Toxicology Program) Draft Technical Report (1994c). Toxicology and carcinogenesis studies of nickel sulfate hexahydrate in F344/N rats and B6C3F_1 mice. NTP TR 454, NIH publication No. 94-3370.

Olsen, L. and Jonsen, J. Whole body autoradiography of ^{63}Ni in mice throughout gestation. *Toxicology*, 12:165-172 (1979).

Rice, J.M. Transplacental carcinogenesis. In: *Developmental Toxicology*, edited by Kimmel, C.A. and Buelke-Sam, J., pp. 191-212, Raven Press, New York (1981).

Schroeder, H.A.; Balassa, J.J.; and Vinton, W.H. Chromium, lead, cadmium, nickel and titanium in mice: effect on mortality, tumors and tissue levels. *J. Nutr.* 83: 239-250 (1964).

Schroeder, H.A.; Mitchener, M.; and Nason, A.P. Life-term effects of nickel in rats: survival, tumors, interactions with trace elements and tissue levels. *J. Nutr.* 104: 239-243 (1974).

Schroeder, H.A. and Mitchener, M. Life-term effects of mercury, methyl mercury, and nine other trace metals on mice. *J. Nutr.* 105: 452-458 (1975).

Sunderman, F.W.; McCully, K.S.; and Rinehimer, L.A. Negative test for transplacental carcinogenicity of nickel subsulfide in Fischer rats. *Res. Commun. Chem. Pathol. Pharmacol.* 31: 545-554 (1981).

APPENDIX B

U.S. AND FOREIGN MINING, MILLING, AND SMELTING OPERATIONS

TABLE 2-2: U.S. AND FOREIGN MINING, MILLING, AND SMELTING OPERATIONS

FACILITY AND LOCATION	MATERIAL PROCESSED	TYPE OF PROCESS	PRODUCT
Bindura Zimbabwe	Sulfide ore	Electrolytic	Cathode
Cerro Matoso Colombia	Laterite ore	Pyrometallurgical	FeNi shot
China Jinchuan	Sulfide ore	Electrolytic	Cathode
Codemin Brazil	Laterite ore	Pyrometallurgical	FeNi shot
Cubaniquel Punta Gorda, Cuba Nicaro, Cuba	Laterite ore	Hydrometallurgical Ammonical leach	NOS*
Empress Nickel (Rio Tinto) Zimbabwe	Sulfide ore	Electrolytic	Cathode
Eramet-SLN Doniambo , New Caledonia Sandouville , France	Laterite ore Sulfide matte	Pyrometallurgical Electrolytic	FeNi shot Cathode
Falconbridge Kristiansand, Norway Bonao, Dominican Republic	Sulfide matte Laterite ore	Electrolytic Pyrometallurgical	Cathode FeNi cones
Fenimak FYROM	Laterite ore	Pyrometallurgical	FeNi
Impala South Africa	Sulfide concentrate	Hyrometallurgical	Briquettes
INCO Sudbury, Canada Thompson, Canada Clydach, Wales	Sulfide ore Sulfide matte oxide sinter	Carbonyl Electrolytic Carbonyl	Pellets, powder, NOS Cathode Pellets, powder
Korea Nickel Korea	Oxide sinter	Pyrometallurgical	Utility Slugs
Korea Nickel Korea	Oxide sinter	Pyrometallurgical	Utility nickel
Larco Greece	Laterite ore	Pyrometallurgical	FeNi shot
Morro do Niquel Brazil	Laterite ore	Pyrometallurgical	FeNI shot
Nippon Yakin Japan	Laterite ore	Pyrometallurgical	FeNi shot
Outokumpu Finland	Sulphide matte	Electrolytic Hydrometallurgical	Cathode Briquettes
Pacific Metals Japan	Laterite ore	Pyrometallurgical	FeNi shot
PT Aneka Tambang Indonesia	Latrerite ore	Pyrometallurgical	FeNi shot
Queensland Nickel Australia	Laterite ore	Hydrometallurgical	Rondelles
Russian Federation Norilsk Severonikel	Sulfide ore Oxide sinter	Electrolytic Carbonyl, Pyrometallurgical	Cathode Pellets, FeNi
Rustenburg South Africa	Sulfide concentrate	Electrolytic	Cathode
Sherritt Gordon Canada	Sulfide concentrate	Hydrometallurgical	Briquettes Powder

TABLE 2-2: U.S. AND FOREIGN MINING, MILLING, AND SMELTING OPERATIONS

FACILITY AND LOCATION	MATERIAL PROCESSED	TYPE OF PROCESS	PRODUCT
Sumitomo Niihama, Japan Hyuga, Japan	Sulfide concentrate Laterite ore	Electrolytic Pyrometallurgical	Cathode FeNi
Taiwan Nickel Taiwan	Oxide sinter	Pyrometallurgical	Utility nickel
Tocantins Brazil	Sulfide ore	Electrolytic	Cathode
Tokyo Nickel Japan	Oxide sinter	Pyrometallurgical	NOS
Ukraine Republic	Laterite ore	Pyrometallurgical	FeNi
Western Mining Australia	Sulfide ore	Hydrometallurgical	Briquettes

APPENDIX C

REVIEW OF THE MANUSCRIPT BY ANDERSEN *ET AL.* TITLED "EXPOSURE TO NICKEL COMPOUNDS AND SMOKING IN RELATION TO INCIDENCE OF LUNG CANCER AMONG NICKEL REFINERY WORKERS."

The Andersen *et al.* (1996) Study of Kristiansand Workers and the Assessment of Carcinogenic Risks Associated with Exposure to Soluble Nickel

S.K. Seilkop [Statistician for the ICNCM study (1990)]

August 20, 1996

The recent paper by Andersen *et al.* (1996) has stimulated concern in the European community over the impending regulatory classification of nickel chloride. This concern primarily relates to the lung cancer risks in Kristiansand nickel refinery workers. In considering the classification of nickel chloride, as well as the reclassification of nickel sulfate that has also been proposed, it is important to address several questions: 1) What is our current understanding of the mechanisms of carcinogenicity for nickel compounds, particulary those that are water soluble? 2) Do the Andersen *et al.* lung cancer results from Kristiansand workers differ from those of previous studies of these workers or from those found in other epidemiologic cohorts exposed to soluble nickel? 3) How does the Andersen *et al.* smoking analysis contribute to our understanding of human health risks associated with soluble nickel exposure? and 4) How do we use the available data in assessing and managing cancer risks associated with exposure to these compounds ?

1. What is our current understanding of the mechanisms of carcinogenicity for nickel compounds?

In evaluating cancer risks associated with nickel compounds, the importance of considering all of the available epidemiologic, animal study, and mechanistic information was recognized by the International Committee on Nickel Carcinogenesis in Man, which concluded its report (ICNCM, 1990) with the following statement:

"Other information to help refine our understanding of human health risks associated with nickel exposure is on the horizon. For example, animal carcinogenesis studies using inhalation as the route of exposure for nickel subsulfide, high temperature nickel oxide, and nickel sulfate hexahydrate are currently underway, and it will be of great interest to see if they support our findings. In addition, future work that improves our understanding of the mechanisms of nickel carcinogenesis may help to unify and explain the results of our findings in conjunction with animal experimentation."

Since the time of the ICNCM report, the animal experimentation to which this passage refers has been completed. Two-year bioassays of rats and mice conducted by the National Toxicology program of the U.S. showed distinctly different carcinogenic risks for the three nickel compounds (NTP, 1996a, 1996b, 1996c). While nickel subsulfide gave clear evidence of producing increased rates of lung tumor incidence in both sexed of rats (but not mice), the results for nickel oxide were less definitive, with some evidence of increased lung cancer rates in both sexes of rats, and equivocal evidence in female mice. For nickel sulfate hexahydrate, there was no evidence to suggest that the compound was carcinogenic in either rats or mice.

The complete pathological examinations and evaluations of lung lavage fluid that were performed in these studies provide insight into disparities between nickel compounds with respect to acute toxicity and inflammatory response. While there is evidence of cytotoxicity and inflammatory response in lung tissue for all three nickel compounds, the severity of this response appears to be related to nickel solubility (Benson *et al.*, 1989). Thus, when effects produced by each compound at equivalent nickel aerosol concentrations are compared, they are consistently strongest for nickel sulfate and weakest for nickel oxide, while nickel subsulfide exhibits an intermediate response.

Persistent inflammatory and cytotoxic responses often induce cell proliferation, which has also been observed in the lung epithelia of rodents exposed to either nickel oxide or nickel subsulfide (Oberdörster *et al.*, 1995). While cell proliferative response has not yet been examined in animals exposed to water soluble nickel compounds, it is likely to be even stronger, given the evidence of a higher degree of acute respiratory toxicity for nickel sulfate than for nickel oxide or subsulfide. Cell proliferation contributes to the carcinogenic process, as it is required to convert repairable DNA lesions into non-repairable mutations, whether these DNA lesions are directly produced by a compound, or whether they are indirect lesions produced by oxygen radicals (Swenberg, 1995). Cell proliferation is also involved in clonal expansion of initiated cell populations, thereby increasing the probability of a second mutational event that leads to malignancy. Thus, through increased cell proliferative activity, nickel compounds can potentially act as promoters of genotoxic events induced by the nickel compounds themselves or by other substances. Based on the disparities in respiratory toxicity of the compounds, the strength of this promotional effect would appear to be directly related to water solubility, with the soluble compounds likely to be the strongest promoters.

The potential for nickel to be delivered to the target cell nucleus also appears to vary with solubility. Endocytosis is considered to be the primary mechanism for delivery of nickel compounds to the cell nucleus (Costa et al., 1981) . Although nickel oxide and nickel subsulfide are readily endocytized, soluble compounds are not (Sunderman Jr., *et al.*, 1987). One might surmise that solubility through diffusion would afford greater access to the cell nucleus. However, the Ni(II) ion is believed to cross the cell membrane using the Mg(II) ion transport system, and in the cell must compete with millimolar levels of Mg(II). Furthermore, soluble nickel compounds such as nickel sulfate are rapidly cleared (Benson *et al.* 1995). Thus, an efficient mechanism for delivery of soluble nickel compounds to the cell nucleus does not appear to exist. Because of the absence of a delivery mechanism, soluble nickel would not be expected to be a carcinogen *per se*; this has been corroborated in the NTP two-year bioassays for nickel sulfate (NTP, 1996c). The cell proliferative activity that it is likely to induce would, however, place it in the category of a potential cancer promoter. In contrast, less soluble compounds that are readily endocytized and which also have been demonstrated to induce increased cell proliferation would be more likely to act as complete carcinogens.

2. Does the Andersen et al. (1996) paper indicate a lung cancer response that differs from that which was found by the ICNCM or from those found in other epidemiologic cohorts?

When the Kristiansand workers were studied by the International Committee on Nickel Carcinogenesis in Man (ICNCM, 1990), they were followed-up through 1984. Lung and nasal cancer risks were based primarily on mortality. Andersen *et al.* have extended the follow-up of these workers through 1993 and conducted their analysis on the incidence of lung and nasal cancer cases (some of whom may still be living). As much of the cohort was hired prior to 1960, a considerable amount of follow-up (at least 24 years) had already occurred for much of the cohort at the time of the ICNCM report, the results that Andersen *et al.* obtained would be expected to be similar to those reported by the ICNCM.

This is indeed the case. The ICNCM evaluated lung and nasal cancer risk both on a process basis (*e.g.*, electrolysis vs. roasting and smelting) and like Andersen *et al.*, with respect to cumulative exposure to different nickel compounds. The Committee's report concluded that workers in two areas (electrolysis, roasting and smelting) had increased lung and nasal cancer risks. However, it also indicated that the electrolysis department, where the predominant nickel exposure was to soluble compounds, had appreciably higher lung cancer risks than the roasting and smelting area, where the primary exposure to nickel was in oxidic and sulfidic form. The ICNCM interpreted the results of its cumulative exposure analysis (summarized in Figure 1a) as giving evidence of an association between soluble nickel and lung cancer risk, but it also discussed the possibility of an interaction between soluble and oxidic nickel exposure. This was suggested by the disparities in risk attributable to "high" cumulative exposure to oxidic nickel ($\geq$15 mg Ni m^3 year) levels at different levels of cumulative exposure to soluble nickel. In particular, at the lowest and highest levels of soluble nickel exposure, differences in risk between "high"

and "low" oxidic nickel exposure were relatively small (<1.25-fold increase in high relative to low). However, when the exposures to soluble nickel were more moderate (5-14 mg Ni m^3 year), there was an appreciably larger (more than two-fold) increased risk for men exposed to high levels of oxidic nickel relative to the risk in men exposed to low oxidic levels. Based on additional evidence of an interaction that was suggested by cross-classified cumulative exposure analyses of workers at the Clydach (Wales) refinery before 1930, the ICNCM concluded that "there was an indication that soluble nickel in some way played a role in accentuating risk associated with exposure to other nickel compounds." This response is clearly consistent with the animal and mechanistic experimental evidence suggesting a promotional role for soluble nickel.

The results that Andersen *et al.* obtained were characterized with a Poisson regression model, based on cross-classified soluble and oxidic nickel exposures. Although this model is useful in showing general trends in response relative to each of these forms of nickel individually, it does not provide sufficient detail to explore the possibility of an interaction between soluble nickel and oxidic nickel. In presentations made in a NiPERA sponsored research workshop (June, 1996) and at a European Union meeting on the classification and labelling of dangerous substances, Andersen provided the summary data (Table 1) on which the Poisson regression model was based. The dose-response function derived from these data (Figure 1b) has the same general features as those found by the ICNCM (Figure 1a). Both sets of data provide evidence that soluble nickel plays a strong role in the induction of lung cancer in Kristiansand workers, but there is also evidence that its role is one of enhancement of other risks. In particular, there is the same indication that the level of risk associated with "high" oxidic nickel exposure is dependent on the level of soluble nickel exposure. Specifically, for cumulative soluble nickel exposure of less than 1 mg/m^3 year, the difference between SIR's for "high" and "low" oxidic nickel exposure is approximately 1.0; however, for cumulative soluble nickel exposure of 1-4 mg/m^3 year, this difference is more than twice as large. The Andersen *et al.* data also suggest a similar enhancement in lung cancer risk at the highest level of soluble nickel exposure($\geq$15 mg/m^3 year, thus providing additional support to the ICNCM hypothesis of an interaction between soluble and oxidic nickel.

As well as lending strength to this hypothesis, the Andersen *et al.* study facilitates a better understanding of the dose-response function for soluble nickel at low levels of oxidic nickel exposure. While the ICNCM study did not find evidence of increased lung cancer risk in men exposed to less than 5 mg/m^3 year soluble nickel and less than 15 mg/m^3 year oxidic nickel, this result was highly uncertain because of the small amount of available data (Figure 1a, 50% confidence interval for SMR$\approx$ 50 - 400). The evaluation of lung cancer risk was also complicated by its apparent enhancement in unexposed workers (SMR=183). The additional follow-up in the Andersen *et al.* study provides risk estimates that are more precise, thereby permitting a clearer definition of the dose-response curve and improved insight with respect to the ICNCM results. In particular, the SIR for workers with less than 1 mg/m^3 year cumulative exposure to both soluble and oxidic nickel (Table 1, SIR= 1.8, 95% C.I.= 1.6-2.0, depicted as "unexposed" in Figure 1b) is virtually identical to that of unexposed workers in the ICNCM report. The estimated lung cancer risk for workers with the same level of soluble nickel exposures and less than 15 mg/m^3 year oxidic nickel is slightly higher, but statistically comparable (SIR=2.0, 95% CI=1.9-2.3). At this level of oxidic nickel exposure, there is also no evidence of additional risk when soluble exposure is increased to 1-4 mg/m^3 year (SIR=2.1, 95% C.I.=1.9-2.5). Thus, the Andersen et al. study produces evidence to suggest that exposure to soluble nickel at 1-4 mg/m^3 year and oxidic nickel of less than 15 mg/m^3 year did not add to the apparent background lung cancer risk in Kristiansand workers. The increased SIR's in workers exposed to these levels of soluble and oxidic nickel are probably not work-related, and are due to other causes. The most likely of these, cigarette smoking, is discussed in Section 3 below.

Evidence of an absence of increased lung cancer risks for low-level soluble nickel exposure (and low levels of oxidic nickel) in Kristiansand workers is consistent with evidence from other epidemiologic data. Specifically, the ICNCM found little, if any evidence to suggest that Clydach workers who were exposed to low levels of soluble nickel and oxidic nickel (SMR=196) had increased lung cancer risk relative to those workers who were unexposed (SMR=166). The contention that low level soluble nickel in the

absence of high nickel oxide exposure does not produce increased lung cancer risk is also supported by the ICNCM analysis of INCO's Port Colborne electrolysis workers. For the 2,747 men in this operation who had less than five years in sintering operations, there was no evidence of a gradient of lung cancer risk with years worked in electrolysis, and only a marginally increased risk overall (SMR=137). That these workers showed a much lower risk than those at Kristiansand was attributed by the ICNCM to either a lower level of soluble nickel than found at Kristiansand and/or a seven-fold lower level of insoluble exposure. The Andersen *et al.* study facilitates a higher degree of understanding of the Port Colborne data. Based on the environmental estimates for the two facilities, nearly all of the Port Colborne workers would be found in the lowest two exposure categories of the bottom curve in Figure 1b. Thus, the absence of risk in the Port Colborne workers is consistent with the Kristiansand dose-response function derived from the Andersen *et al.* data.

3. How does the Andersen et al. smoking analysis contribute to our understanding of human health risks associated with soluble nickel exposure?

One of the difficulties with the ICNCM study, as well as other epidemiological investigations of lung cancer in nickel workers, has been the absence of smoking data. Smoking is widely accepted as the principal cause of lung cancer. Furthermore, "blue collar" workers, such as those in nickel refineries, typically have higher smoking prevalence than the general population. Thus, using national mortality (or incidence) rates to calculate Standardized Mortality Ratios (or Standardized Incidence Ratios) for such workers often results in upwardly biased estimates of lung cancer risk.

In the ICNCM study, there was evidence that Kristiansand workers smoke more than the general population. This was based on an SMR of 183 in workers who had no exposure to nickel. Unfortunately, the "ever" vs."never" smoking data in the Andersen *et al.* paper do not provide a complete picture of the smoking patterns of Kristiansand workers relative to that of the general population. Based on the expected number of cases in Table 6, the primary information about smoking patterns that can be inferred is that the proportion of "ever" smokers in the cohort is approximately 80%, which is similar to the rate of "ever" smokers during the same period in other industrialized countries (*e.g.*, Canada). The paper does not, however, have information about the prevalence and intensity of cigarette consumption during the follow-up period. If this information was available, it could be used to evaluate the validity of assuming that the increased lung cancer risk in unexposed Kristiansand workers found by ICNCM is due to greater cigarette consumption. Nonetheless, Andersen *et al.* found that workers exposed to both soluble and oxidic nickel at less than 1 mg/m^3 year had a significantly increased SIR of 1.8 ($p<0.01$), which was virtually identical to the SMR for unexposed workers examined by the ICNCM. That this increase is not due to nickel exposure is supported by a statistically significant increased lung cancer risk (SIR=2.0, two-sided p=0.011) for workers with less than 15 years since first exposure in the refinery (Andersen *et al.*, Table 3). Since the latency period for lung cancers is generally believed to be more than 15 years, cancers identified earlier than 15 years from time of first employment at Kristiansand are likely to have been induced by smoking that was begun prior to when workers started working at the refinery. Thus, there is evidence of a two-fold smoking-induced increase in the background lung cancer risk for Kristiansand workers. The same two-fold increase in workers with exposure to low levels of nickel therefore appears to be attributable to workers' smoking habits, and not to nickel exposure.

The primary importance of the Andersen *et al.* analysis of lung cancer and smoking behavior in nickel workers is in its contribution to the understanding of the mechanisms for nickel carcinogenesis. Most importantly, the strong evidence of synergy between cigarette smoking and nickel exposures in inducing lung cancer provided by Andersen *et al.* corroborates experimental evidence suggesting that nickel compounds act as "promoters" of genotoxic events arising from exposure to initiators or complete carcinogens (such as tobacco smoke).

The animal evidence that soluble nickel is likely to be a more powerful promoter than insoluble compounds is also supported by the Andersen *et al.* data. The absence of evidence of carcinogenicity in the NTP study of nickel sulfate hexahydrate suggests that humans exposed to soluble nickel alone would not

experience increased lung cancer risk. The Andersen *et al.* Kristiansand data appear to contradict this, with evidence of a dose-related risk above 5 mg/m^3 year cumulative soluble nickel exposure, even when the level of oxidic nickel exposure nickel is low (figure 1b). Furthermore, there is an indication of a soluble nickel dose-related increase when cumulative oxidic nickel exposure is less than 1 mg/m^3 year (Table 1). Unlike laboratory animals in the NTP studies, however, workers' lungs at the Kristiansand refinery were exposed to soluble nickel in the presence of a substance that has been demonstrated to contain powerful initiators (*i.e.*, tobacco smoke). Thus, the association between soluble nickel exposure and lung cancer risk found in Kristiansand workers conforms to the anticipated promotional response. Furthermore, the weaker lung cancer response to oxidic nickel exposure might also be anticipated. There is an increasing body of evidence that that carcinogenic process is extremely sensitive to changes in cellular kinetics, particularly through enhanced cell turnover induced by cytotoxicity. As discussed above, oxidic nickel exhibits less toxicity in lung tissue than soluble nickel, and would therefore not be expected to enhance the effect of smoking as strongly as would soluble nickel.

It should be noted that while the lung cancer response to soluble nickel exposure in Kristansand workers can be reasonably attributed to the promotion of smoking-induced cancers, and this response might be elicited by other nickel compounds as well, it is clearly not the only response that is likely to be associated with exposure to less soluble compounds. The animal, mechanistic study, and epidemiologic data suggest that these compounds induce genetic or epigenetic effects as well as cell proliferative activity. However, in conjuction with the epidemiologic data, animal and mechanistic study data strongly suggest that lung cancer risks associated with soluble nickel exposure are dependent upon the concomitant presence of other substances with the potential to initate the carcinogenic process.

4. Implications for Risk Assessment and Risk Management

The evidence that water soluble nickel compounds are promoters rather than complete carcinogens has important implications for regulatory and industrial personnel engaged in risk assessment and risk management for these compounds. As a respiratory irritant, soluble nickel might be expected to produce an inflammation dose-response curve which exibits strong non-linearity or a threshold effect. This is based on the fact that one of the roles of respiratory epithelial cells is to maintain a protective barrier against inhaled pathogens and toxic chemicals. When exposed to low levels of a respiratory toxicant, epithelial cells are capable of performing this protective role. However, at sufficiently high concentrations the epithelial barrier can be breached, thereby exposing underlying cells to risk of direct exposure to air contaminants, and the influx of inflammatory cells which may release powerful chemical mediators (Butterworth *et al.*, 1995). This "all or nothing" response induces a strongly non-linear or threshold dose-response function for cell proliferative activity. As a consequence, a promoter acting through increased cell proliferation exhibits either non-linearity or a threshold in dose-related enhancement of cancer risks associated with exposure to complete carcinogens (such as tobacco smoke). The epidemiologic data from Kristiansand refinery workers provides evidence of such a threshold in lung cancer risk for men exposed to soluble nickel at relatively low levels of oxidic nickel exposure (lower curve in Figure 1b). The understanding of the likely origin of this threshold response provides confidence that if airborne concentrations of soluble nickel are sufficiently low, there is no substantive risk that exposure to these compounds will promote the carcinogenic activity of other hazards.

References

Andersen, Aa., Engeland, A.., Berge, S.R., Norseth, T. (1996) Exposure to nickel compounds and smoking in relation to incidence of lung and nasal cancer among nickel refinery workers. Unpublished manuscript.

Benson, J.M., Burd, D.G., Cheng, Y.S., Hahn, F.F., Haley, P.J., Henderson, R.F., Hobbs, C.H., Pickrell, J.A., and Dunnick, J.K. (1989). Biochemical responses of rats and mous lung to inhaled nickel compounds. *Toxicology* 57: 255-266.

Benson, J.M., Barr, E.B., Bechtold, W.E., Cheng, Y.S, Dunnick, J.K., Eastin, W,E., Habbs, C.H., Kennedy, C.H., Maples, K.R. (1995). Fate of inhaled nickel oxide and nickel subsulfide in F344/N Rats. *Inhalation Toxicology* 6:167-183.

Butterfield, B.E., Conolly, R.B., Morgan, K.T. (1995) A stragegy for establishing mode of action of carcinogens as a gude for approaches to risk assessments. *Cancer Letters* 93:129-146.

Costa, M., Abbracchio, M.P., Simmons-Hanson, J. (1981). Factors influencing the phagocytosis, neoplastic transformation, and cytotoxicity of particulate nickel compounds in tissue culture systems. *Toxciol. Appl. Pharmacol.* 60: 313-323.

ICNCM (1990). Report of the International Committee on Nickel Carcinogenesis in Man, *Scandinavian Journal of Work, Environment & Health*, Volume 16, number 1(special issue), February, 1990, 82 pp.

NTP (1996a). NTP Technical Report on the Toxicology and Carcinogenicity of Nickel Oxide (CAS No. 1313-99-1) in F344/N Rats and B6C3F_1 Mice (Inhalation Studies). NTP Technical Report 451.

NTP (1996b). NTP Technical Report on the Toxicology and Carcinogenicity of Nickel Subsulfide (CAS No. 12035-72-2) in F344/N Rats and B6C3F_1 Mice (Inhalation Studies). NTP Technical Report 453.

NTP (1996c). NTP Technical Report on the Toxicology and Carcinogenicity of Nickel Sulfate Hexahydrate (CAS No. 10101-97-0) in F344/N Rats and B6C3F_1 Mice (Inhalation Studies). NTP Technical Report 454.

Oberdörster, G., Baggs, R.B., Finkelstein, J. (1995). Pulmonary retention and effects of inhaled NiO and Ni_3S_2 in rats and mice:indicators of maximum tolerated dose: Fifteh COMTOX Symposium on Toxicology and Clinical Chemistry of Metals. Vancouver, BC Canada (July, 1995), page 26.

Sunderman, F.W. Jr., Hopfer, S.M., Knight, J.A., McCully, K.S., Cecutti, A.G, Thornhill, P.G., Conway, K., Miller, C., Patierno, S.R., Costa, M. (1987). Physicochemical characteristics and biological effects of nickel Oxides. *Carcinogenesis* 8(2): 305-313.

Swenberg, J.A. (1995). Bioassay design and MTD setting: old methods and new appproaches. *Reg Toxicol. and Pharm.* 21:44-51.

Table 1

(Results from the Norwegian study, not included in the paper)

NUMBERS OF NEW CASES OF LUNG CANCER AMONG 4902 MALE NICKEL REFINERY WORKERS, BY CUMULATIVE EXPOSURE TO NICKEL COMPOUNDS; FOLLOW-UP, 1953-92

Soluble nickel compounds (mg/m^3)	Cumulative Exposure to Oxidic Nickel (mg/m^3)									
	< 1		1 - 4		5 - 14		≥ 15		TOTAL	
	O	SIR	O	SIR	O	SIR	O	SIR	O	SIR
< 1	40	1.8**	2	1.4	17	3.3**	33	2.9**	92	2.3**
1-4	15	2.6**	13	1.8	2	2.0	5	4.6*	35	2.3**
5-14	3	4.3	10	2.2*	8	6.5**	1	1.8	22	3.1**
≥ 15	1	13.3	16	5.6**	27	8.2**	8	9.2**	52	7.3**
Total	59	2.0**	41	2.6**	54	5.0**	47	3.4**	201	2.9**

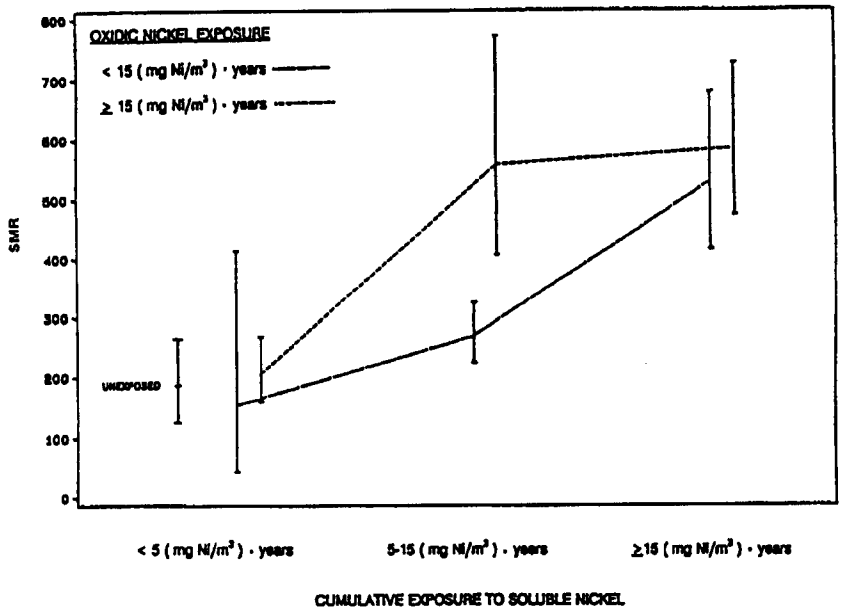
O, number of observed cases; SIR, standardized incidence ratio.

* $p < 0.05$

** $p < 0.01$

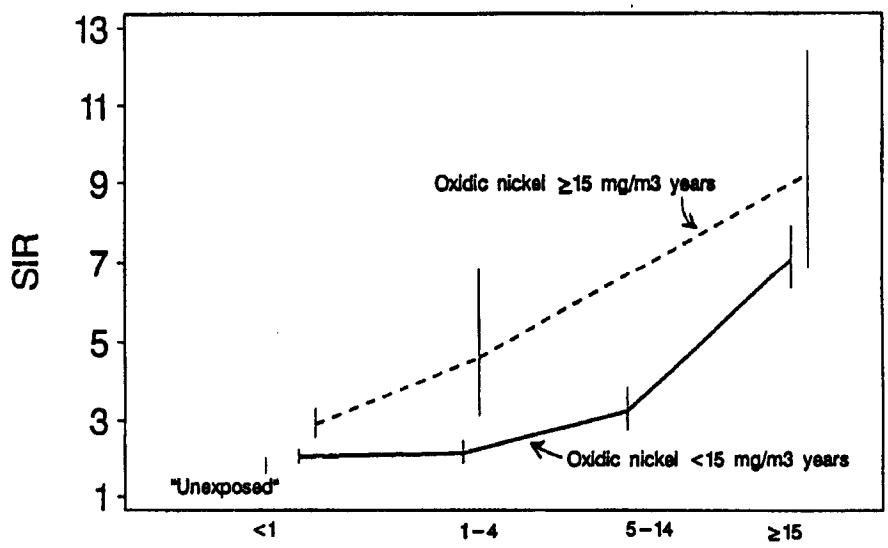
Figure 1
Lung Cancer Risks[1] by Cumulative Exposure to Soluble and Oxidic Nickel

ICNCM



(a)

Andersen et al.[2]



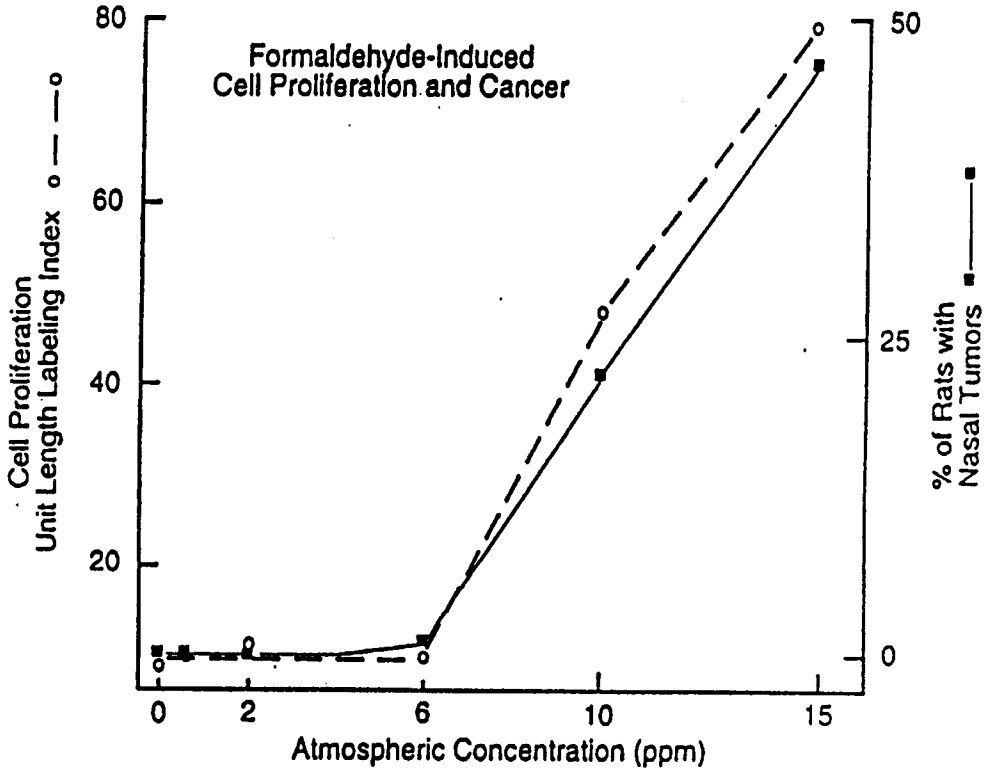
(b)

1 SMR's and SIR's plotted with 50% confidence limits.

2 Note: SIR for category with cumulative soluble nickel exposure of 5-14 mg/m^3 years and cumulative oxidic nickel ≥15 mg/m^3 year is not plotted as it is based on a single lung cancer case.

Figure 2

Threshold Response in Cell Proliferation and Tumor Incidence
for Rats Exposed to Formaldehyde (Butterworth *et al.*, 1995)



APPENDIX D

DETAILED COMMENTS REGARDING THE FINNISH REFINERY STUDIES

CONCOMITANT EXPOSURES - SULFURIC ACID MIST AND INSOLUBLE NICKEL

While the authors of the RoC Background Document have noted the presence of sulfuric acid mist in the Outokumpu tankhouse during the period critical to the development of the lung and nasal cancers, not enough attention is given to this detail.[1] IARC (1992) classified inorganic mists containing sulfuric acid as being carcinogenic to humans. This conclusion was based on studies that showed an association of sulfuric acid mist exposures with nasal sinus, laryngeal, and lung cancers in workers in various manufacturing operations. The nasal sinus cancers are of particular interest in that they occurred mainly in workers manufacturing isopropanol, a compound that has not been demonstrated to be carcinogenic in laboratory animals through any routes of exposure tested (inhalation, subcutaneous injection, skin painting, and diet) (Weil *et al.*, 1952; Van Esch, 1960; NIOSH, 1976; Burleigh-Flayer *et al.*, 1997).[2] Others, in addition to IARC, have also concluded that nasal cancers seen in isopropanol workers result from the strong acid process utilized in such plants (Solskolne *et al.*, 1984; Lynch *et al.*, 1979).

It is essential to note this, since it appears that the generation of strong acid mists in the Outokumpu tankhouse has always been a problem that the company has strived to control.[3] Communications from Outokumpu indicate that strong inorganic acid mists containing sulfuric acid measured in 1966 ranged from 0.2 to 1.2 mg/m^3, with a mean and median of 0.6 mg/m^3. The mean concentration for such samples taken over the 1970s was even higher (0.8 mg/m^3). Although such concentrations of mists containing sulfuric acid were believed to be safe back then, the safety of such concentrations may be questionable in light of the IARC Report that showed an association of respiratory cancers with sulfuric acid mists at or below such concentrations.

As noted in the RoC Background Document, there is also evidence that nickel exposures in the electrolytic part of the refinery for much of the period relevant to the induction of respiratory cancer were to both soluble and insoluble forms of nickel. The critical exposure period for the induction of both the nasal and lung cancers seen in these workers would have been in the 1960s through the early 1970s when the nickel refinery was first put into operation and engineering "bugs" were being eliminated from the system.[4]

With respect to soluble nickel, documentation from Outokumpu indicates that during the first 15 years of the refinery's operation, only 11 stationary samples were taken in the tankhouse and this occurred during a two-day period in November of 1966. Both the mean and median concentrations of these samples were 0.5 mg Ni/m^3; concentrations up to 0.8 mg Ni/m^3 were reported. No samples were taken previous to this point, and the next set of samples taken in the tankhouse were not until 1976. With respect to insoluble nickel exposures, concentrations in grinding and leaching (a part of the electrolysis hall until 1973) were reported to be as high as 2 mg Ni/m^3. Therefore, it is clear that exposures in the 1960s-early 1970s were, at least in a number of instances, higher than those taken between 1979-1981. The use of the 1979-1980 exposure measurements (reported to be below 0.5 mg Ni/m^3) as the basis for the analysis of cancer mortality by the authors of the study is, therefore, potentially misleading.

---

1 It should be noted that the authors of the paper failed to mention the presence of these acid mists.

2 Nasal cancers have also been seen in phosphate fertilizer workers exposed to sulfuric acid mists (Hagmar *et al.*, 1991).

3 Anode hoods and gas channels to draw off oxygen and electrolyte mists were installed when the refinery opened, but problems were encountered when salts accumulated in the channel. Mist suppression was reported as being problematic. Because of these problems, the hoods were removed in 1976 and the anodes were enclosed in polyester bags in an attempt to prevent misting from the surface of the electrolyte. In a continuing effort to reduce mists, the bags were replaced with polyurethane balls in 1980.

4 The two workers with "confirmed" nasal cancers had retired by 1982. Nasal cancer latency in nickel refinery workers in other cohorts has been observed to be at least 15 years, with some latency periods of 30 years or more being reported. Therefore, if nickel was the causative agent of the nasal cancers in the Finnish workers, it would have been the early exposures (1960s) that contributed to these cancers. Likewise, with respect to lung cancer, it appears that all 6 lung cancers observed in the nickel refinery workers came from workers with 20+ years of latency, raising the possibility that these cancers, too, occurred in workers who were exposed to higher concentrations of soluble and insoluble nickel compounds, as well as acid mists, in the early years of the refinery's operation.

ESTABLISHMENT OF NASAL CANCER CASES - TIMING, DIAGNOSIS, OTHER WORK HISTORY

Nasal cancer is sufficiently rare that a single spontaneous case in a small cohort can produce significance by conventional statistical criteria (p=0.05), and two cases will achieve a very high level of significance (p» 0.001). However, to infer a causal link from such a small number of cases, without a more thorough examination of them is simply not good science. This is particularly true in an instance such as this where the total number of workers presenting with nasal cancers is small and, therefore, it would not be difficult to examine these cancers on a case-by-case basis. Specifically, as nasal cancer is known to be associated with other occupations (*e.g.*, carpentry work), it is important to investigate previous work experience. An evaluation of the timing of the occurrence of the cancer relative to this work experience assists in developing a more firmly grounded assessment of the most likely origin of the cancer.

Information obtained from the company on the four potential nasal cancers reported reveals that two of the workers were previously employed in carpentry work prior to their work in the nickel refinery at Harjavalta. While this does not rule out a possible role for nickel exposures contributing to these nasal cancers, it does call into question the precise etiologic cause of the cancers. The possibility that the carpentry work could have caused these nasal cancers should be noted, particularly because nasal cancers have such a long latency period. Further, the type of work that these workers were involved in at the refinery should be noted as certain jobs (*e.g.* maintenance, cleaning) are likely to result in exposures that are higher than the norm.

In addition, while it is not unreasonable for the authors to note the nasopharyngeal cancer seen in one female worker relative to her exposure to nickel in the refinery, the fact that the origin of this cancer (nose or pharynx) is uncertain may also be of some relevance, as other nasal cancers in nickel refinery workers have originated in the nasal sinuses. It would be helpful to reexamine the pathology of all the nasal sinus cancers observed in the Finnish cohort to determine their precise origin. If most of the purported " nasal sinus" cancers prove to be of uncertain origin (*i.e.* possibly pharyngeal in nature), the results from this cohort would differ from any other nickel cohort studied (see attached Table). This would require a much more rigorous examination of the exposures and processes involved in this refinery (*e.g.* electrowinning versus electrolyses) that might set it apart from other refineries. All of these factors should be discussed in the RoC Background Document as they may have a profound influence on inferences drawn from this study.

COMPARABILITY OF NASAL CANCERS IN THE FINNISH STUDIES TO THOSE OF OTHER STUDIES

In the Norwegian studies, while there was some evidence linking nasal cancer to soluble nickel exposures, the evidence was much stronger for oxidic nickel (Andersen *et al.*, 1996). In contrast to the situation at Outokumpu, no nasal cancers have occurred in Kristiansand workers first employed since 1956, nor have there been any excess nasal cancer in workers who have been employed in Clydach during a comparable time period.[5] It is also worth noting that many of the Kristiansand electrolysis workers had exposures to soluble nickel that were higher than those reported at Outokumpu. Furthermore, the exposures of sulfuric acid mist were lower at Kristiansand than at Outokumpu. This again raises the possibility that sulfuric acid mist or the combination of soluble nickel with sulfuric acid mists induced the nasal cancers in Outokumpu workers. In short, the very large nasal cancer risk in Outokumpu workers is inconsistent with that found in other nickel refinery workers with a comparable (or higher) degree of soluble nickel exposure. This weakens the evidence that soluble nickel was the putative

[5] Since 1950, two nasal cancer deaths have occurred in Clydach workers (Draper *et al.*, 1994). One was in a worker recruited in 1964 at the age of 63 who worked for the company for less than two years; it is questionable whether his nasal cancer can be attributed to his brief employment at Clydach. The second nasal cancer occurred in a worker who was hired in 1953 and worked for the company for 11 years. This worker was involved in cleaning one of the old Mond reducing towers being used for experimental nickel powder production. In this activity, his exposure to inorganic nickel compounds would have been considerably higher than that of the other workers, and he had no soluble nickel exposure as it was not believed to have been present in the Mond reducers.

agent, and strongly suggests that exposures to other carcinogenic agents (*e.g.*, sulfuric acid mist) may have played a causal or contributory role.

THE POTENTIAL ROLE OF SMOKING IN THE FINNISH LUNG CANCERS

As seen in the Norwegian cohort, a higher prevalence of smoking in the Finnish refinery workers could be a possible explanatory factor for the increased lung cancer rates seen in this study. Although the increased rate of lung cancer in non-refinery workers at Outokumpu (SIR=1.48 for those with 20+ years of latency) is not elevated statistically, it suggests the possibility of increased smoking prevalence in the workforce at Outokumpu that would bias the reported results. The contention that this is true is strengthened by the significantly elevated SIR for lung cancer in Outokumpu smelter workers (SIR=2.00) and the absence of duration of exposure-related lung cancer response in these workers (or those in the refinery).

Epidemiologists generally believe that relative risks for lung cancer in excess of approximately 1.5 are unlikely to be due to differential patterns of cigarette smoking. Evidence from Kristiansand nickel refinery workers, however, challenges this view. Specifically, workers with little, if any nickel exposure at Kristiansand exhibited enhanced lung cancer risks (SMR=183 in Report of the ICNCM, 1990; SIR=1.8 in Andersen *et al.*, 1996) which can be logically attributed to the abnormally high proportion of smokers at Kristiansand (»80%) that can be derived from Table 6 of Andersen *et al.* (1996). A similar smoking prevalence in Outokumpu workers would inflate the true baseline lung cancer risk to the level observed in smelter workers, which is statistically consistent with that of unexposed workers. At a background rate comparable to that of Kristiansand (SIR=1.8), the increase in lung cancer risk in the Finnish refinery workers can be reasonably attributed to chance alone (p=0.11). Furthermore, there is no compelling statistical evidence to differentiate lung cancer risk in smelter workers from that of refinery workers (p=0.27 for workers with 20+ years latency, p=0.08 overall).

To adequately interpret the increased lung cancer incidence at Outokumpu, it is essential to obtain a better characterization of cigarette smoking prevalence in the Finnish workers. The Kristiansand study strongly suggests that smoking prevalence in refinery workers may far exceed that of a national or even regional reference population which is used to derive SIRs. It is not at all far-fetched to assume that a similar situation exists with the Outokumpu workers. Consequently, the lung cancer SIR's in this study may be severely upwardly biased, and this possibility should be addressed in the discussion of the results of the study.

EVIDENCE THAT SOLUBLE NICKEL IS A PROMOTER, AND NOT A COMPLETE CARCINOGEN

The lack of elevated tumor rates in the NTP animal bioassay on nickel sulfate (NTP, 1996) supports the theory that soluble nickel, alone, is not a complete carcinogen and that the effects of soluble nickel seen in humans may be due to its promotional characteristics. This reflects the ICNCM Report's conclusion that soluble nickel enhanced the respiratory cancer risks associated with exposure to other nickel compounds (both sulfidic and oxidic forms), and should be clearly stated in the RoC Background Document.

The role of soluble nickel as a promoter rather than a complete carcinogen is derived from epidemiologic data from the Clydach refinery before 1930, comparisons of Port Colborne to Kristiansand electrolysis workers, in Kristiansand workers alone, (see above comments), as well as the most recent theories regarding the carcinogenic mechanisms and bioavailability of different nickel species (Oller *et al.*, 1997). A discussion of this information needs to be included in this report to allow the reader to examine the results of the analysis of the Finnish workers in the context of the current scientific understanding of soluble nickel's role in carcinogenesis.

REFERENCES

Burleigh-Flayer, H.; Garman, R.; Neptun, D.; Bevan, C.; Gardiner, T.; Kapp, R.; Tyler, T.; and Wright, G. (1997). Isopropanol vapor inhalation oncogenicity study in Fischer 344 rats and CD-1 mice. *Fund. Appl. Toxicol.,* 36, 95-111,

Draper, M. H.; Morgan, L. G.; Metcalf, L.M.; Duffus, J. H.; Park, M. V.; Johns, P. (1994). Study of the evolution of the nickel refinery processes and the chemical nature of some of their historical process materials and contemporary environmental dusts at the INCO nickel refinery works at Clydach, Wales, U.K. Report to Nickel Producers Environmental Research Association.

Hagmar, L.; Bellander, T.; Andersson, C.; Linden, K.; Attewell, R.; and Moller, T.(1991). Cancer morbidity in nitrate fertilizer workers. *Int. Arch. Occup. Environ. Health,* 63, 63-67.

Lynch, J.; Hanis, N.M.; Bird, M.G.; Murray, K. J.; and Walsh, J.P. (1979). An association of upper respiratory cancer with exposure to diethyl sulfate. *J. occup. Med.,* 21, 333-341.

NIOSH (1976). Criteria for a recommended standard. Occupational exposure to isopropyl alcohol. p.54. U.S. DHEW, PHS, CED, Rockville, MD.

NTP (1996c). NTP Technical Report on the Toxicology and Carcinogenicity of Nickel Sulfate Hexahydrate (CAS No. 10101-97-0) in F344/N Rats and B6C3F_1 Mice (Inhalation Studies). NTP Technical Report 454.

Solskolne, C.; Zeighami, E.; Hanis, N.; Kupper, L.; Herrmann, N.; Amsel, J., Mausner J. ; and Stellman, J. (1984). Laryngeal cancer and occupational exposure to sulfuric acid. *Am. J. Epidemiol.*, 120,358-369.

Van Esch, G. (1960). Suitability of a rapid test for carcinogenic properties of chemical compounds with the aid of a promoter substance. *Verslag. Medel. Betreffende Volksgezondheid.*, 186-189.

Weil, C.; Smyth, H.; Nale, T. (1952), Quest for a suspected industrial carcinogen. *Industrial Hygiene and Occuaptional Medicine,* 5, 535-547.